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TITLE: Preparation of Chemicals and Bulk Drug Substances for the U.S. Army Drug Development Program

PRINCIPAL INVESTIGATOR: Jaroslav F. Novotny, Ph.D.

CONTRACTING ORGANIZATION: Starks Associates, Inc.
Buffalo, NY 14213

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PREPARED FOR: Commander
U. S. Army Medical Research and Materiel Command
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Buffalo, NY 14213

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A broad spectrum of chemical compounds for U. S. Army Drug Development Program were synthesized during this period. Nineteen candidate drugs were delivered. New synthetic methods were designed for eight products transmitted to WRAIR.

The purity of all target compounds and intermediates were rigorously checked by a series of physical and chemical tests. Methods have been developed for compounds not previously recorded in the chemical literature. The cost for raw materials and labor were kept under strict control by minimizing the turn-around-time for each requested material.

Continued on the next page.

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Infectious disease related compounds and intermediates, chemical defense related compounds and intermediates.

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The following target compounds have been synthesized during this period: WR99210 prodrug, amide from β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure); $N^1$-3,4-dichlorophenyl-$N^5$-isopropyl-diguanide, hydrochloride; 1-(3,4-dichlorophenyl)-3-(1-isopropyl-4,5-dioxo-2-imidazolidinylidene)guanidine; 6-cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine; 1,3,5-triazine-2-acetic acid, 4-[(3,4-dichlorophenyl)amino]-1,6-dihydro-6-imino-1-(1-methylethyl)-, methyl ester; (*S*)-4-ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3,6,10-trione; (*S*)-4-ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3,6,10-trione; 1(2*H*)-acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-; *L*-glutamic acid, N-[N-[ethoxyhydroxy-phosphinyl]-*L*-phenylalanyl]-, trilithium salt; *L*-glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, diammonium salt; *L*-glutamic acid, N-[N-[methoxyhydroxy-phosphinyl]-*L*-phenylalanyl]-, trilithium salt; 2-(guanin-7-yl)ethyl 2-hydroxyethyl sulfide; N-(2-hydroxyethyl)-N-[2-(7-guaninyl)ethyl]methylamine; O-[[(1*R*)-N-[N-(phenylmethoxy-carbonyl)glycyl]-1-aminoethyl]methoxyphosphinyl]-(*R*)-lactic acid, methyl ester; O-[[(1*R*)-N-[N-(phenylmethoxycarbonyl)-glycyl]-1-aminoethyl]methoxyphosphinyl]-(*R*)-lactic acid, lithium salt; O-[(*L*)-1-[[N-(phenylmethoxycarbonyl)glycyl]-amino]ethyl]hydroxyphosphinyloxy]-*L*-lactic acid, dilithium salt.

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## I SUMMARY

A broad spectrum of chemical compounds for U. S. Army Drug Development Program were synthesized during this period. Nineteen candidate drugs were delivered. New synthetic methods were designed for eight products transmitted to WRAIR.

The purity of all target compounds and intermediates were rigorously checked by a series of physical and chemical tests. Methods have been developed for compounds not previously recorded in the chemical literature. The cost for raw materials and labor were kept under strict control by minimizing the turn-around-time for each requested material.

The following target compounds have been synthesized during this period: WR99210 prodrug, amide from $\beta,\beta$,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure); $N^1$-3,4-dichlorophenyl-$N^5$-isopropyl-diguanide, hydrochloride; 1-(3,4-dichlorophenyl)-3-(1-isopropyl-4,5-dioxo-2-imidazolidinylidene)guanidine; 6-cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine; 1,3,5-triazine-2-acetic acid, 4-[(3,4-dichlorophenyl)amino]-1,6-dihydro-6-imino-1-(1-methylethyl)-, methyl ester; (*S*)-4-ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3,6,10-trione; (*S*)-4-ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3,6,10-trione; 1(2*H*)-acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-; *L*-glutamic acid, N-[N-[ethoxyhydroxy-phosphinyl]-*L*-phenylalanyl]-, trilithium salt; *L*-glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, diammonium salt; *L*-glutamic acid, N-[N-[methoxyhydroxy-phosphinyl]-*L*-phenylalanyl]-, trilithium salt; 2-(guanin-7-yl)ethyl 2-hydroxyethyl sulfide; N-(2-hydroxyethyl)-N-[2-(7-guaninyl)ethyl]methylamine; O-[[(1*R*)-N-[N-(phenylmethoxy-carbonyl)glycyl]-1-aminoethyl]methoxyphosphinyl]-(*R*)-lactic acid, methyl ester; O-[[(1*R*)-N-[N-(phenylmethoxycarbonyl)-glycyl]-1-aminoethyl]methoxyphosphinyl]-(*R*)-lactic acid, lithium salt; O-[(*L*)-1-[[N-(phenylmethoxycarbonyl)glycyl]-amino]ethyl]hydroxyphosphinyloxy]-*L*-lactic acid, dilithium salt.

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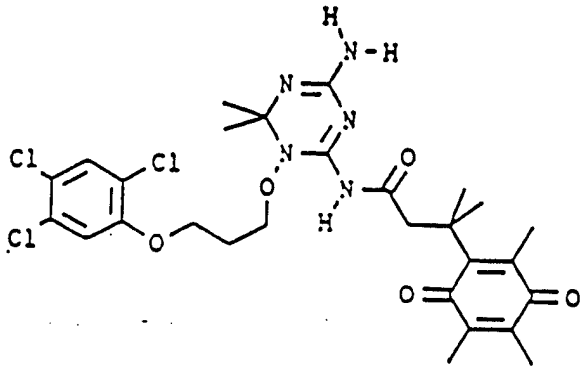
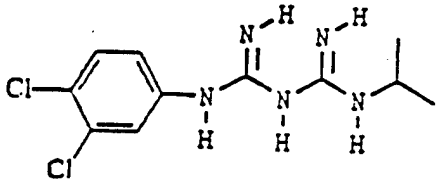
Jaroslav F Novotny 20/12/96

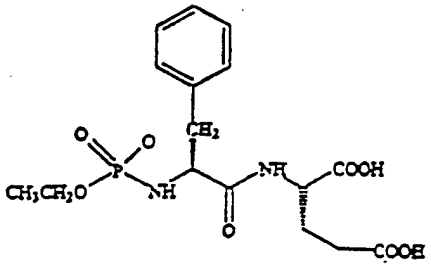
Jaroslav F. Novotny, PI / Date

III. CUMULATIVE LIST OF REQUESTED TARGET COMPOUNDS DELIVERED TO WALTER REED ARMY INSTITUTE OF RESEARCH FROM DECEMBER 1, 1995 TO NOVEMBER 30, 1996

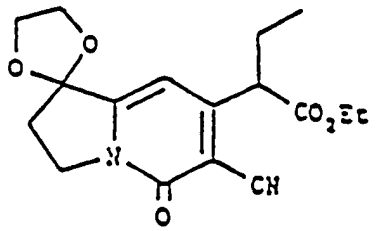
| | Compound | Cumulative No.* |
|---|---|---|
| |  | |
| 1. | WR99210 Prodrug; amide from β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure) | 1214 |
| |  | |
| 2. | $N^1$-3,4-Dichlorophenyl-$N^5$-isopropyldiguanide, hydrochloride | 1215 |

*Additional information may be found in the Cumulative List on page 7, this report.

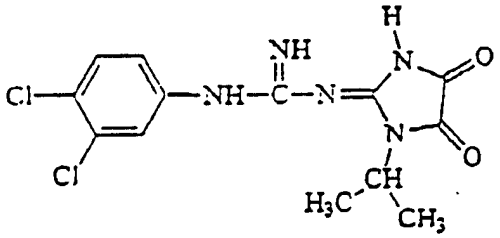
| Compound | Cumulative No. |
|---|---|



3. L-Glutamic acid, N-[N-[ethoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt — 1216



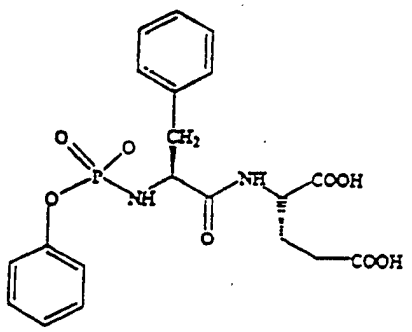
4. 6-Cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine — 1217



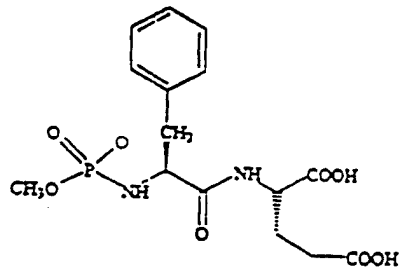
5. 1-(3,4-Dichlorophenyl)-3-(1-isopropyl-4,5-dioxo-2-imidazolidinylidene)guanidine — 1218

Compound

Cumulative No.

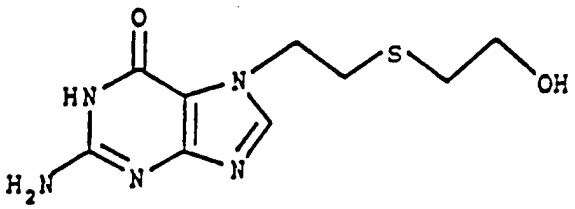


6. L-Glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, diammonium salt 1219

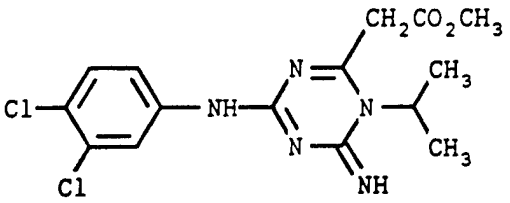


7. L-Glutamic acid, N-[N-[methoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt 1220

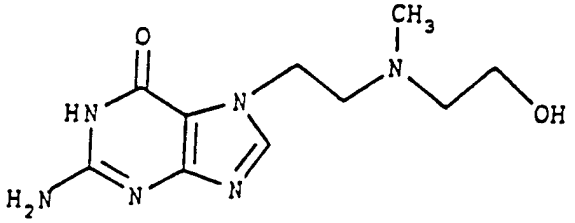
| Compound | Cumulative No. |
|---|---|



8. 2-(Guanin-7-yl)-ethyl 2-hydroxyethyl sulfide — 1221

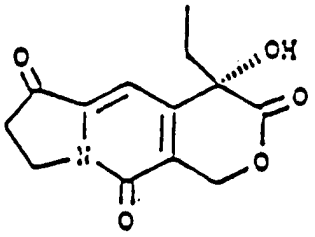


9. 1,3,5-Triazine-2-acetic acid, 4-[(3,4-dichlorophenyl)amino]-1,6-dihydro-6-imino-1-(1-methylethyl)-, methyl ester — 1222

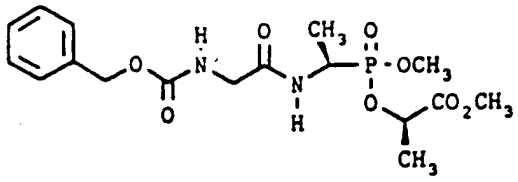


10. N-(2-Hydroxyethyl)-N-[2-(7-guaninyl)ethyl]-methylamine — 1223

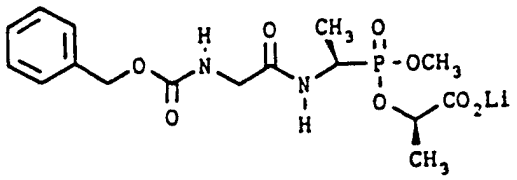
| Compound | Cumulative No. |
|---|---|



11. (*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxy-pyrano[3,4-*f*]indolizine-3,6,10-trione — 1224

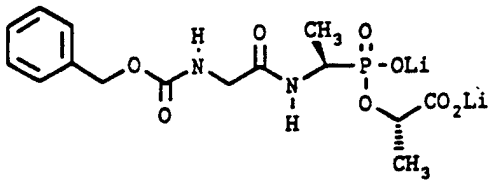


12. O-[[(1*R*)-N-[N-(Phenyl-methoxycarbonyl)glycyl]-1-aminoethyl]methoxy-phosphinyl]-(*R*)-lactic acid, methyl ester — 1225

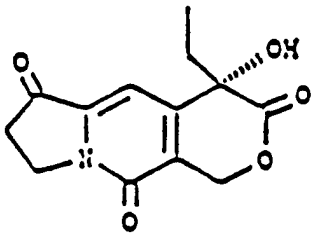


13. O-[[(1*R*)-N-[N-(Phenyl-methoxycarbonyl)glycyl]-1-aminoethyl]methoxy-phosphinyl]-(*R*)-lactic acid, lithium salt — 1226

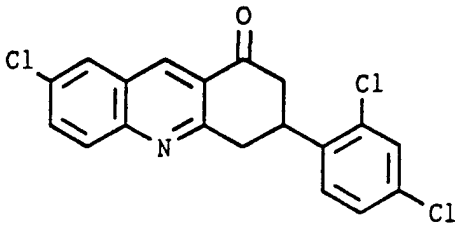
| Compound | Cumulative No. |
|---|---|



14. O-[(*L*)-1-[[N-(Phenylmethoxycarbonyl)glycyl]amino]ethyl]hydroxyphosphinyloxy]-*L*-lactic acid, dilithium salt — 1227



15. (*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3,6,10-trione — 1228



16. 1(2*H*)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)- — 1229

## IV. CUMULATIVE LIST OF COMPOUNDS COMPLETED AND DELIVERED TO WALTER REED ARMY INSTITUTE OF RESEARCH (WRAIR) FROM DECEMBER 1, 1995 TO NOVEMBER 30, 1996

The previous Cumulative List covering the period from July 1, 1965 to June 30, 1973 may be found in Starks Associates, Inc. Final Comprehensive Report dated June 30, 1973 pages 54-97, Contract No. DA-49-193-MD-2751. The list covering the period from July 1, 1973 to September 28, 1979 may be found in Starks Associates, Inc. Final Comprehensive Report dated September 1979, pages 82-241, Contract No. DAMD17-73-C-3159. The list covering the period from September 29, 1979 to September 14, 1983 may be found in Starks Associates, Inc. Final Comprehensive Report dated September 1983, pages 56-163, Contract No. DAMD17-79-C-9170. The list covering the period from September 15, 1983 to March 14, 1989 may be found in Starks Associates, Inc. Final Comprehensive Report dated March 14, 1989, pages 55-116, Contract No. DAMD17-83-C-3206. The list covering the period from March 15, 1989 to November 30, 1992 may be found in Starks Associates, Inc. Final Comprehensive Report dated November 30, 1992, pages 35-58, Contract No. DAMD17-89-C-9058. The list covering the period from December 1, 1992 to November 30, 1993 may be found in Starks Associates, Inc., Annual Report dated November 30, 1993 pages 4-5, Contract No. DAMD17-93-C-3003. The list covering the period from December 1, 1993 to November 30, 1994 may be found in Starks Associates, Inc., Annual Report dated November 30, 1994 pages 4-5, Contract No. DAMD17-93-C-3003. The list covering the period from December 1, 1994 to November 30, 1995 may be found in Starks Associates, Inc. Annual Report dated January 1996 page 10-16, Contract No. DAMD17-93-C-3003.

| Cumulative No. | Compound | Amount | BN# | WR# | Starks Assoc. Report |
|---|---|---|---|---|---|
| 1211 | 6-Hydroxy-4,4,5,7,8-pentamethylhydrocoumarin | 3.4 g | BN64767 | 279647 | 123 |
| 1212 | β,β,2,4,5-Pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid | 3.3 g | BN64776 | 279690 | 123 |
| 1213 | Succinimidyl β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoate | 2.0 g | BN64785 | 280492 | 123 |

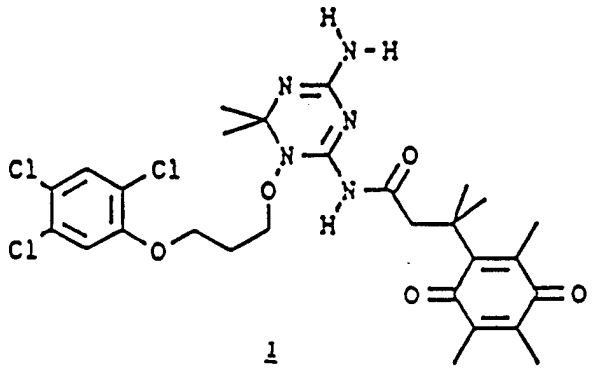
| Cumulative No. | Compound | Amount | BN# | WR# | Starks Assoc. Report |
|---|---|---|---|---|---|
| 1214 | WR99210 Prodrug; amide from β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanonic acid (intended structure) | 1.4 g | BN64794 | 280493 | 123 |
| 1215 | $N^1$-3,4-Dichlorophenyl-$N^5$-isopropyldiguanide, hydrochloride | 29.1 g | BN65111 | 042313 | 123 |
| 1216 | *L*-Glutamic acid, N-[N-[ethoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, | 1.0 g | BN65102 | 280451 | 123 |
| | trilithium salt | 20 mg | BN65488 | 280451 | 123 |
| 1217 | 6-Cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine | 5.3 g | BN65120 | 280157 | 123 |
| 1218 | 1-(3,4-Dichlorophenyl)-3-(1-isopropyl-4,5-dioxo-2-imidazolidinylidene)guanidine | 192.3 g | BN66369 | 182393 | 123 |
| | | 156.6 g | BN66378 | 182393 | 123 |
| 1219 | *L*-Glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, | 700 mg | BN66387 | 280526 | 123 |
| | diammonium salt | 20 mg | BN66396 | 280526 | 123 |
| 1220 | *L*-Glutamic acid, N-[N-[methoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, | 800 mg | BN67473 | 280527 | 123 |
| | trilithium salt | 20 mg | BN67482 | 280527 | 123 |
| 1221 | 2-(Guanin-7-yl)-ethyl 2-hydroxyethyl sulfide | 50 mg | BN70069 | 280607 | 124 |
| 1222 | 1,3,5-Triazine-2-acetic acid, 4-[(3,4-dichlorophenyl)amino]-1,6-dihydro-6-imino-1-(1-methylethyl)-, methyl ester | 150 mg | BN70667 | 280640 | 124 |

| Cumulative No. | Compound | Amount | BN# | WR# | Starks Assoc. Report |
|---|---|---|---|---|---|
| 1223 | N-(2-Hydroxyethyl)-N-[2-(7-guaninyl)ethyl]-methylamine | 800 mg | BN70676 | 280419 | 124 |
| 1224 | (*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxy-pyrano[3,4-*f*]indolizine-3,6,10-trione | 10.2 g | BN72134 | 280463 | 125 |
| 1225 | O-[[(1*R*)-N-[N-(Phenyl-methoxycarbonyl)glycyl]-1-aminoethyl]methoxy-phosphinyl]-(*R*)-lactic acid, methyl ester | 20 mg | BN72143 | 280685 | 125 |
| 1226 | O-[[(1*R*)-N-[N-(Phenyl-methoxycarbonyl)glycyl]-1-aminoethyl]methoxy-phosphinyl]-(*R*)-lactic acid, lithium salt | 20 mg | BN72152 | 280686 | 125 |
| 1227 | O-[(*L*)-1-[[N-(Phenyl-methoxycarbonyl)glycyl]-amino]ethyl]hydroxy-phosphinyloxy]-*L*-lactic acid, dilithium salt | 30 mg<br>1.4 g | BN72634<br>BN78172 | 280693<br>280693 | 125<br>125 |
| 1228 | (*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxy-pyrano[3,4-*f*]indolizine-3,6,10-trione | 20.3 g | BN78921 | 280463 | 126 |
| 1229 | 1(2*H*)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)- | 0.2 g | BN81320 | 280850 | 126 |

## V. DISCUSSION OF RESEARCH AND TARGET COMPOUNDS

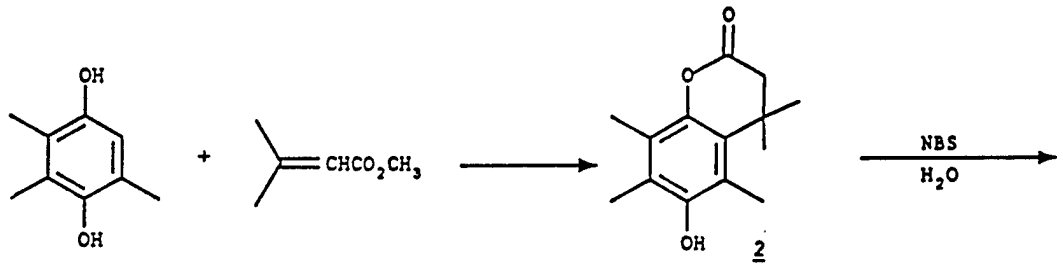
### 1. WR99210 Prodrug; amide from β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure)

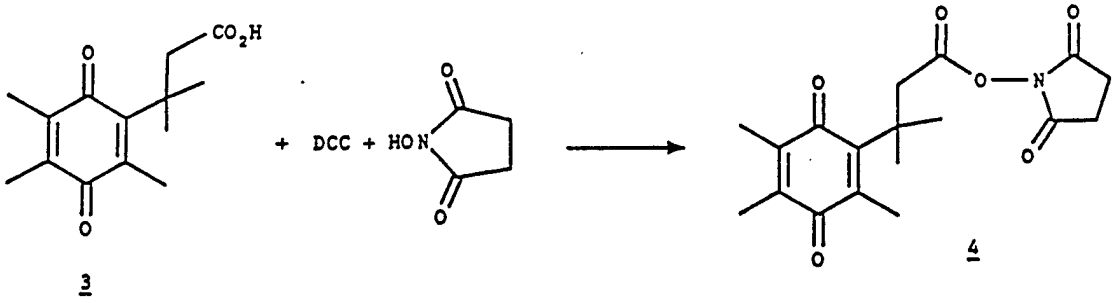
The target compound 1 was prepared

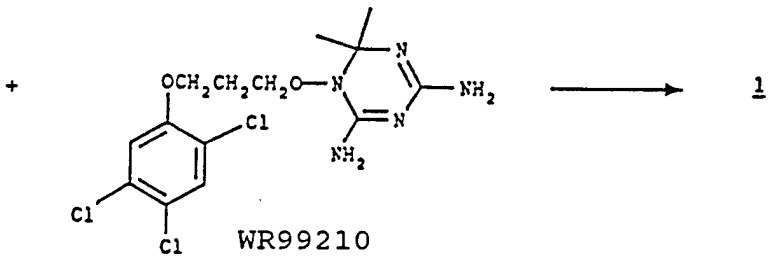


1

by the following sequence of reactions.[1,2]



Trimethylhydroquinone was reacted with methyl 3,3-dimethylacrylate to give hydrocoumarin 2 in 56% yield. Reaction of 2 with N-bromosuccinimide in acetonitrile gave the propanoic acid 3 in 69.9% yield. Reaction of 3 with N-hydroxysuccinimide in the presence of DCC gave the succinimidyl ester 4 in 75.8% yield. Reaction of 4 with WR99210 (free base) gave as one of the products the $N^6$ or $N^4$ amide. The $N^4$ amide shown represents the intended structure. Spectral data (NMR) are not in full accord with the intended structure and further characterization might be necessary. Please note that the NMR spectrum has changed subsequent to shipment.

2. a. *L*-Glutamic acid, N-[N-[ethoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt (9a)
   b. *L*-Glutamic acid, N-[N-[methoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt (9b)
   c. *L*-Glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, diammonium salt (9c)

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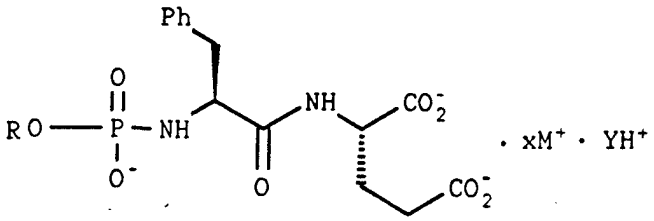
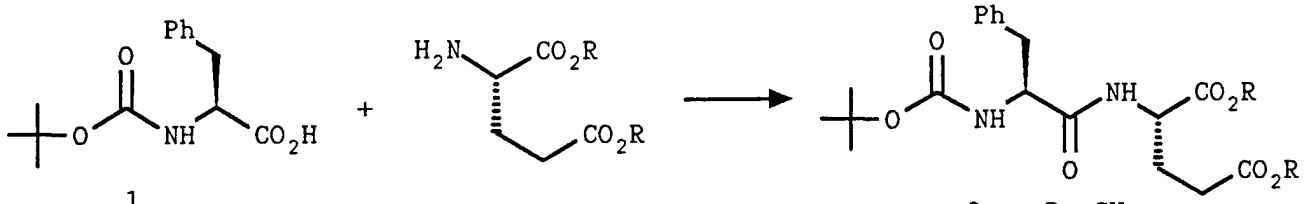



9a : R= $CH_3CH_2$-, x= 3, M= Li, Y= 0
9b : R= $CH_3$-, x= 3, M= Li, Y= 0
9c : R= Ph-, x= 2, M= $NH_4$, Y= 1

Dipeptides 4a and 4b were prepared in 77-91% and 90% yield, respectively, by coupling commercially available *t*-BOC-*L*-phenylalanine (1) with *L*-glutamic acid, dimethyl ester (2a) or dibenzyl ester (2b), using HBTU[3], followed by removal of the *t*-butoxycarbonyl protecting group with trifluoroacetic acid to give 4a and 4b as tosylate salts[4] (98% yield). Intermediates 8a and 8b were prepared in 26% and 18% yield by phosphorylation of 4a with chlorophosphates 7a and 7b, respectively. The protected phosphorylated dipeptides were hydrolyzed with 1.5M LiOH to give 9a and 9b as trilithium salts (88% and 92% yield)[5], containing excess LiCl and $H_2O$.

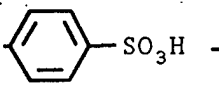
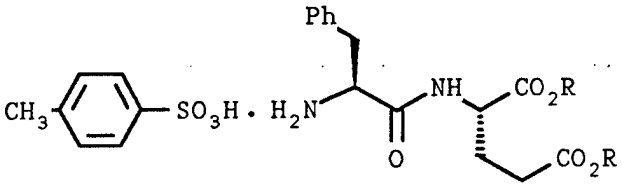
Intermediate 8c was prepared in 23% yield by phosphorylation of 4b with chlorophosphate 7c. The tribenzyl protected phosphorylated dipeptide was debenzylated by transfer hydrogenation[6] with 10% palladium on carbon, employing ammonium formate as the source of hydrogen, to give 9c as a diammonium salt (76% yield), containing excess $H_2O$.

a. 

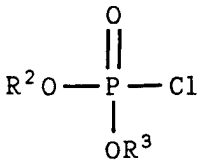
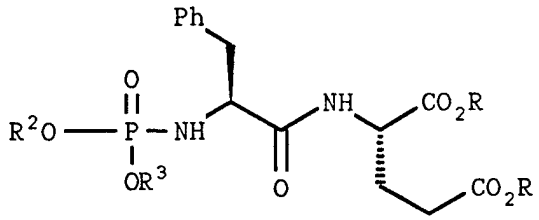
1

2a : R= $CH_3$
2b : R= $CH_2Ph$

3a : R= $CH_3$
3b : R= $CH_2Ph$

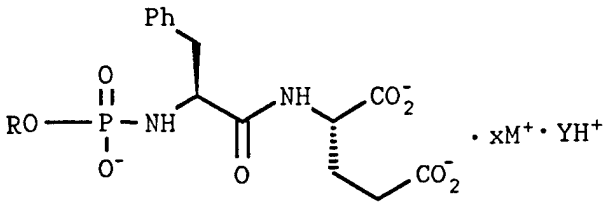
b. 3 + $CF_3COOH$ +  

4a : R= $CH_3$
4b : R= $CH_2Ph$

c. 4a / 4b +  

7a : $R^2$= Et, $R^3$= Ph
7b : $R^2$= $CH_3$, $R^3$= Ph
7c : $R^2$= Ph, $R^3$= $PhCH_2$

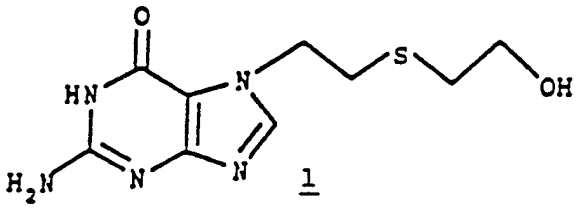
8a : R= $CH_3$, $R^2$= Et, $R^3$= Ph
8b : R= $CH_3$, $R^2$= $CH_3$, $R^3$= Ph
8c : R= $R^3$= $CH_2Ph$, $R^2$= Ph

d. 8a / 8b / 8c  · $xM^+$ · $YH^+$

9a= R= Et, x= 3, M= Li, Y= 0
9b= R= $CH_3$, x= 3, M= Li, Y= 0
9c= R= Ph, x= 2, M= $NH_4$, Y= 1

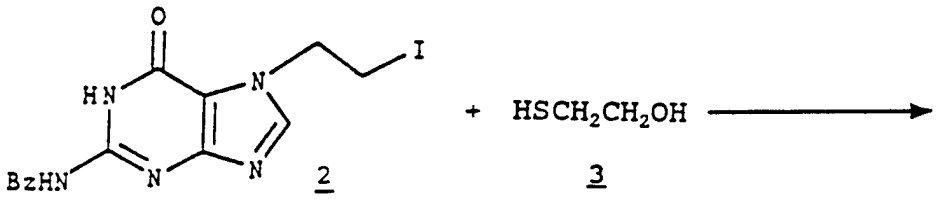
3. 2-(Guanin-7-yl)ethyl 2-hydroxyethyl sulfide (1)

The target compound 1 was prepared

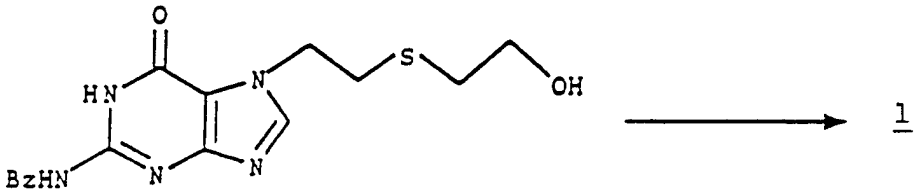


1

from iodoethylpurinone 2 (prepared previously, please see Starks Associates Yearly Report, dated January 1996, p. 172, Contract DAMD17-93-C-3003) and 2-mercaptoethanol (3) using $CH_3ONa$ as the base to give 4 in 94% yield.



2 3



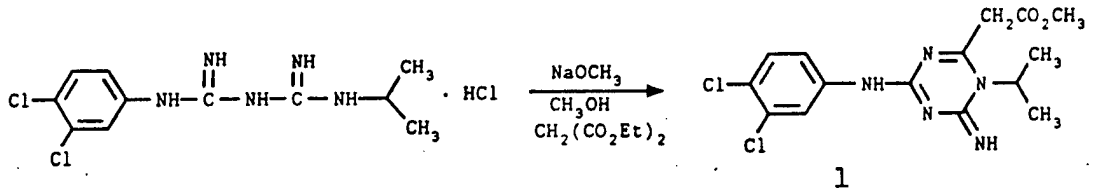
4

Material 4 was deprotected with boiling sodium methoxide in $CH_3OH$ to yield the target material in 99% yield.

4. 1,3,5-Triazine-2-acetic acid, 4-[(3,4-dichlorophenyl)amino]-1,6-dihydro-6-imino-1-(1-methylethyl)-, methyl ester (1)

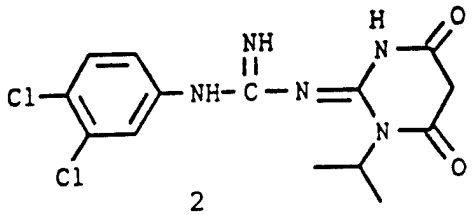
The target compound (tentative structure) was prepared by the following reaction sequence.

Reaction Sequence




1

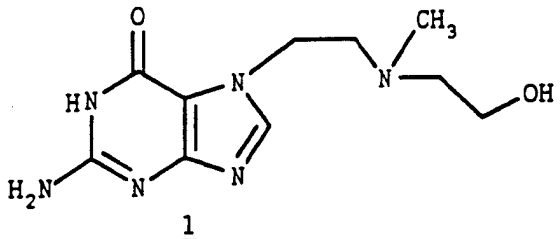
The original intent of this reaction sequence was to produce the pyrimidinone 2, however, none of this material could be isolated under these conditions.




2

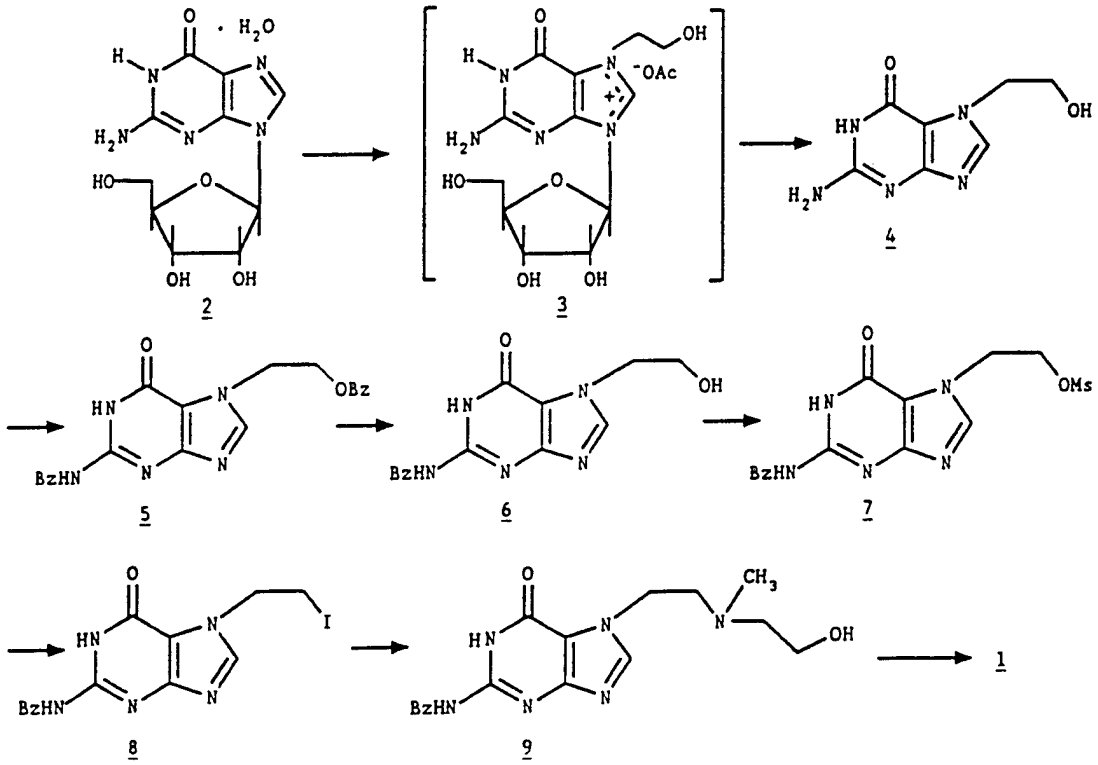
5. N-(2-Hydroxyethyl)-N-[2-(7-guaninyl)ethyl]methylamine (1)

The material 1 was prepared by the



1

following sequence of reactions,[7-10] Guanosine hydrate was

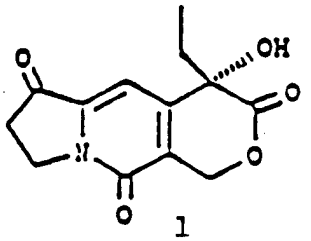


reacted with ethylene oxide in acetic acid and the

intermediate 3 was immediately hydrolyzed to give 4 in 71% yield. The amino- and hydroxy- functions of 4 were protected by the use of benzoyl cyanide to give 5 in 42% yield, then selectively deprotected to yield 6 in 72% yield. Reaction of 6 with MsCl gave 7 in 88% yield. Material 7 was reacted with NaI in acetone to give 8 in 88% yield. Compound 8 was reacted with commercially available 2-methylaminoethanol to give 9 in 84% yield. This material was deprotected with sodium methoxide to give crude target material 1 in 62% yield.

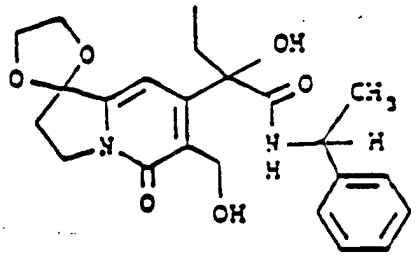
6. (*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]-indolizine-3,6,10-trione (1)

The target compound 1 was prepared

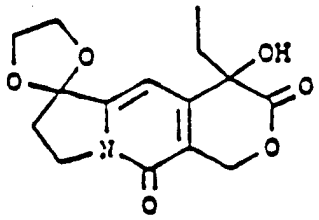


1

from crude intermediate 2 by the procedure shown below.

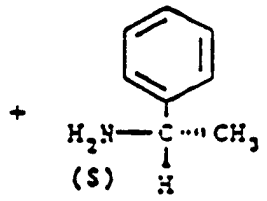


2

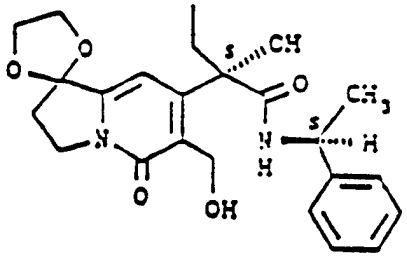


HOAc, Δ 75°

3



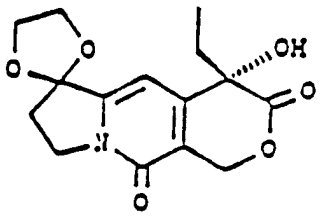
Δ, 70°C



HOAc, Δ 75°

4

and (R,S)-Diastereomer (4a)



5

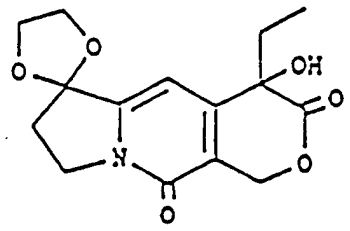
Aq $H_2SO_4$, Dioxane

1

Compound 2 (saved from an earlier synthesis of the target material) was hydrolyzed yielding the racemic ketal 3. The ketal 3 was reacted with (*S*)-(-)-α-methylbenzylamine to give the (*S*,*S*)- and (*R*,*S*)-diastereomers 4 and 4a. These were separated by trituration and crystallization, then hydrolyzed to obtain optically active 5. Material 5 was deprotected to give the target compound 1.

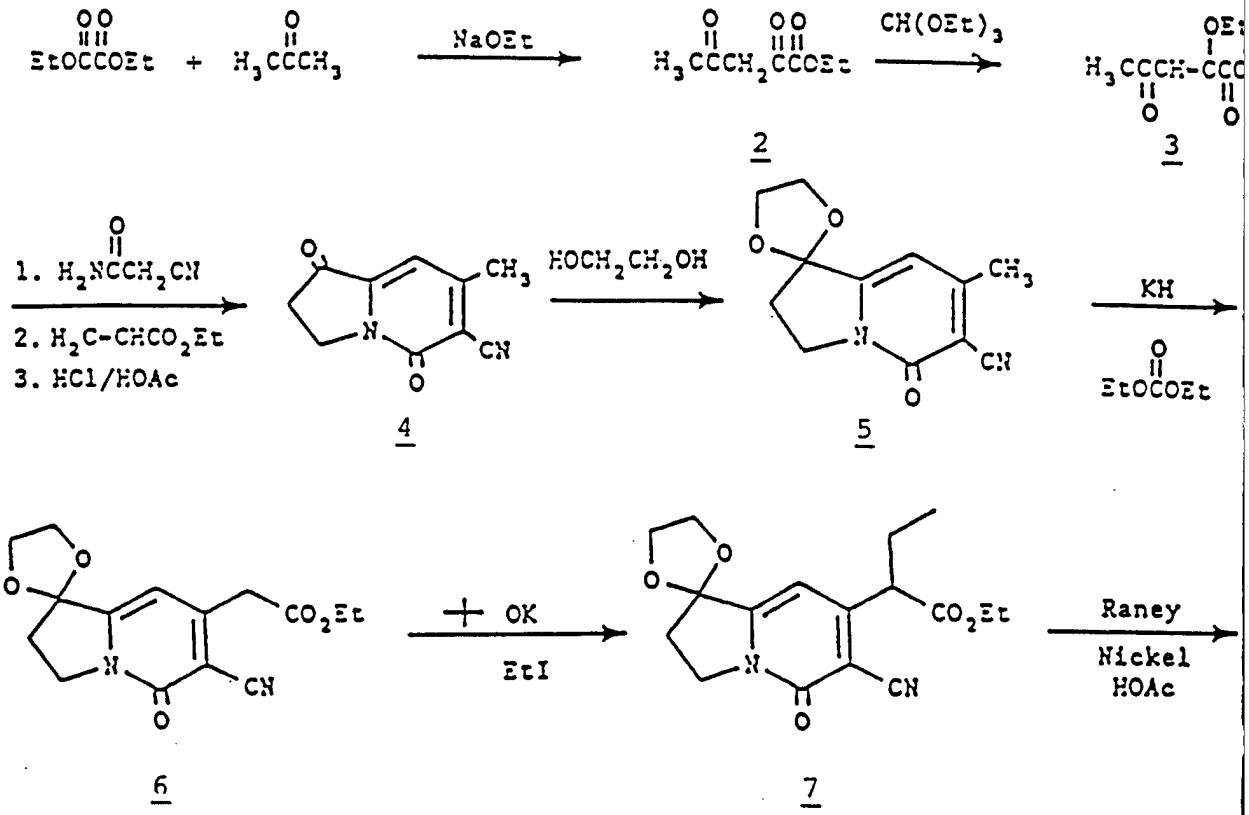
7. (*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]-indolizine-3,6,10-trione (12)

The racemic target ketal 1 was prepared

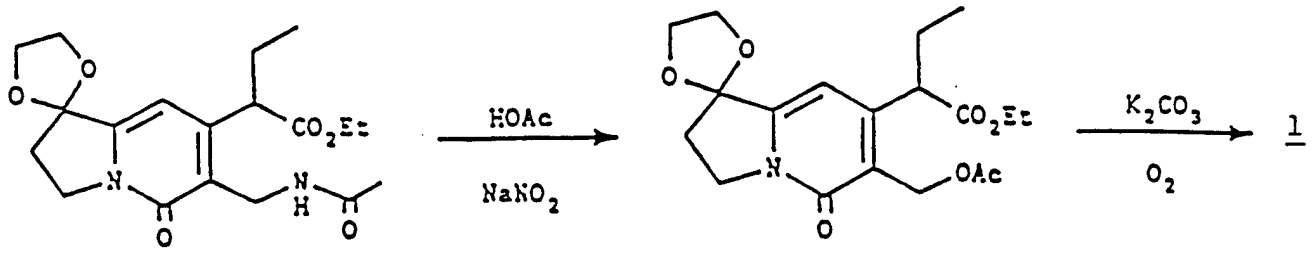


1

by a nine step literature[11-13] procedure shown below.

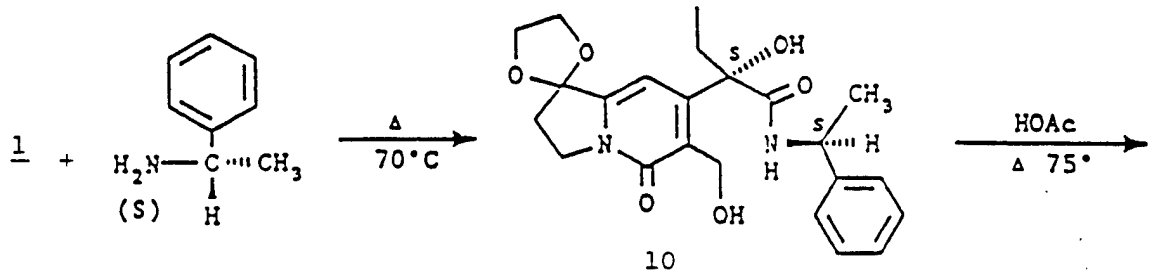



2 3 4 5 6 7



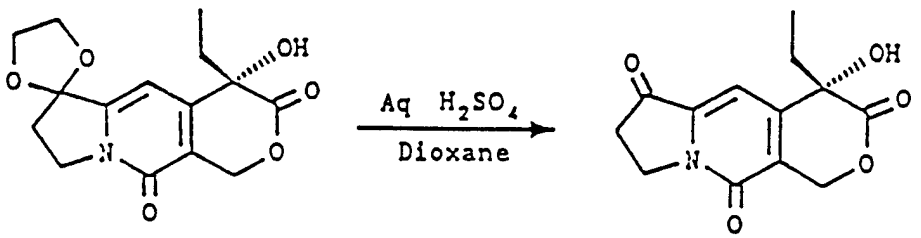

8 9

The racemic ketal 1 was reacted with (*S*)-(-)-α-methylbenzylamine to give the (*S*,*S*)- and (*R*,*S*)-diastereomers 10 & 10a. These were separated by trituration and crystallization then hydrolyzed yielding optically active 11 and (*R*)-enriched 1. Material 11 was deprotected to give the target compound 12.




10

and (R,S)-Diastereomer (10a)




11 12

## VI. RESEARCH AND KNOWN TARGET COMPOUNDS AND INTERMEDIATES COMPLETED AND DELIVERED TO WALTER REED ARMY INSTITUTE OF RESEARCH FROM DECEMBER 1, 1995 TO NOVEMBER 30, 1996

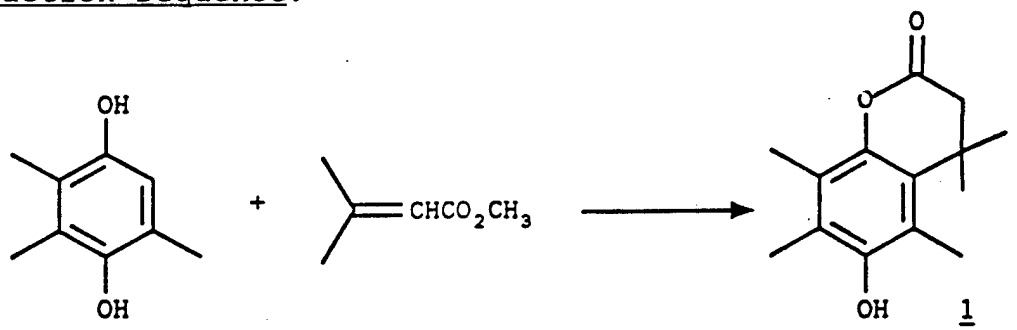
### A. Infectious Disease Related Compounds and Intermediates

#### 1. WR99210 Prodrug; amide from β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure)

The target compound 4 was prepared by the following sequence of reactions. Intermediates 1-3, which are new to the program, were transmitted to WRAIR.

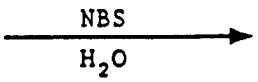
Reaction Sequence:

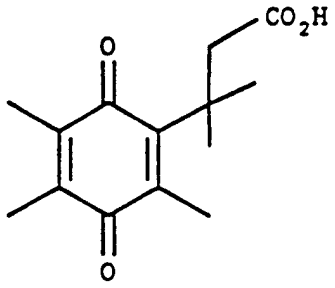
a.




1

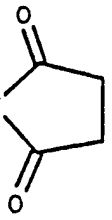
b. 1 +

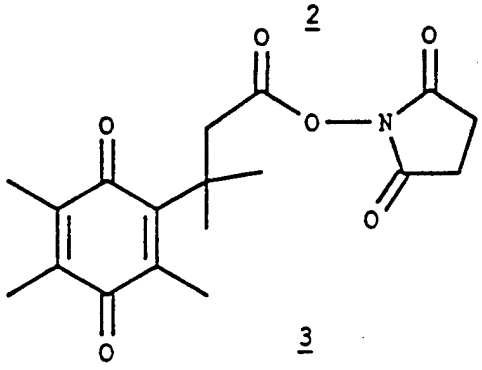



2

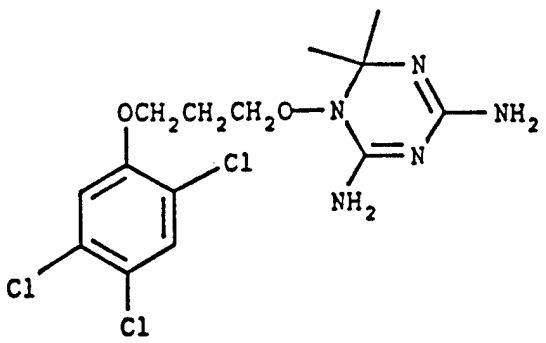
c. 2 + DCC +



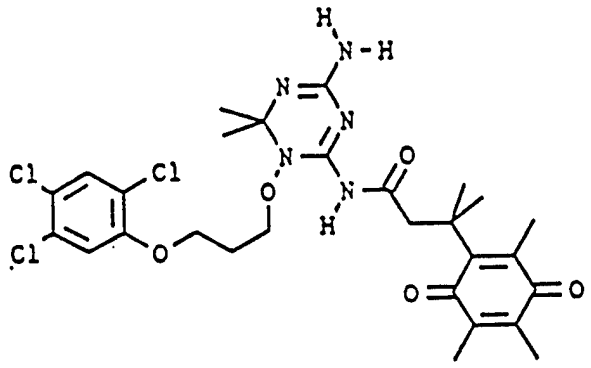

3

d.



WR99210

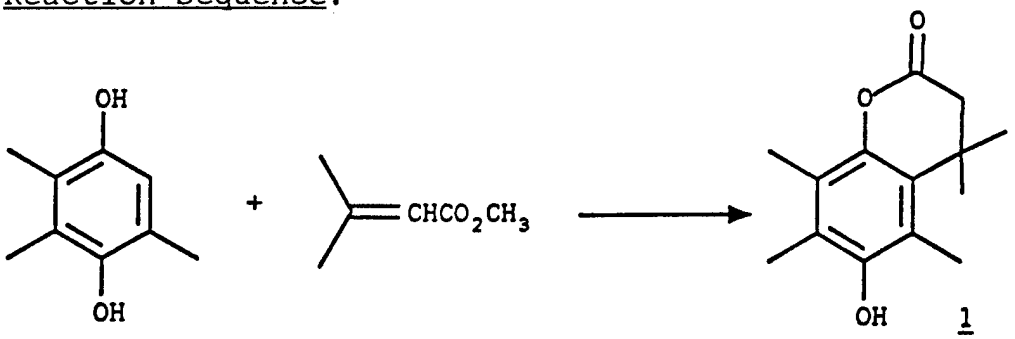
+ 3 ⟶



4

1. 6-Hydroxy-4,4,5,7,8-pentamethylhydrocoumarin (1)

Reaction Sequence:




1

## Experimental

To heated (70°C) methanesulfonic acid (100 mL) was added 10 g (65.7 mmol) of trimethylhydroquinone and 8.5 g (9.8 mL, 74.5 mmol) of methyl 3,3-dimethylacrylate all at once, and the mixture was stored at 70°C for 90 min, cooled, poured into 1.25 L of $H_2O$, then extracted with EtOAc (3 x 500 mL). The extracts were washed with $H_2O$, saturated $NaHCO_3$, and NaCl solutions (500 mL each), then dried ($MgSO_4$). Solvent removal gave the crude lactone as a tan solid (15.5 g). The material was purified by chromatography on a column of silica gel (500 g), packed and eluted with $CH_2Cl_2$. The following fractions were collected (250 mL each): fractions 1-4 contained product and a faster running impurity (4.2 g); fractions 5-7 contained pure product (6.8 g) and fractions 8-14 contained product and a slower running impurity (3.8 g), total recovery 16.3 g. Product from fractions 5-7 (6.8 g) was recrystallized from hexane-EtOAc, yield 3.8 g (55.9% recovery), mp 185-185.5°C, literature[14,15] mp 183-186°C and 186-187°C, respectively. A portion (3.4 g) will be transmitted to WRAIR, (Lot No. NJ14-149-2). Additional product (45 g) was obtained from another reaction. The material was suitable for further transformation.

Anal.

| | | C | H |
|---|---|---|---|
| Calc'd for | $C_{14}H_{18}O_3$ | 71.77 | 7.74 |
| | Found | 71.89 | 7.77 |

Spectral Data

Infrared (Nujol)

Major bands: 3420, 2910, 2840, 1730, 1640, 1600, 1445, 1400, 1365, 1355, 1300, 1280, 1245, 1195, 1170, 1155, 1110, 1070, 1020, 950, 900 $cm^{-1}$.

Ultraviolet (EtOH)

$\lambda_{max}$ 210 nm (sh, log $\varepsilon$ 4.42); 240 nm (sh, 3.69); 288 nm (3.48).

Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 4.72 (s, 1, OH); 2.54 (s, 2, H's at C-3); 2.36 (s, 3, $CH_3$ at C-8); 2.22 (s, 3, $CH_3$ at C-7); 2.19 (s, 3, $CH_3$ at C-5); 1.45 (s, 6, 2 x $CH_3$ at C-4). $D_2O$ exchanges proton at 4.72 ppm.

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - ultraviolet light.

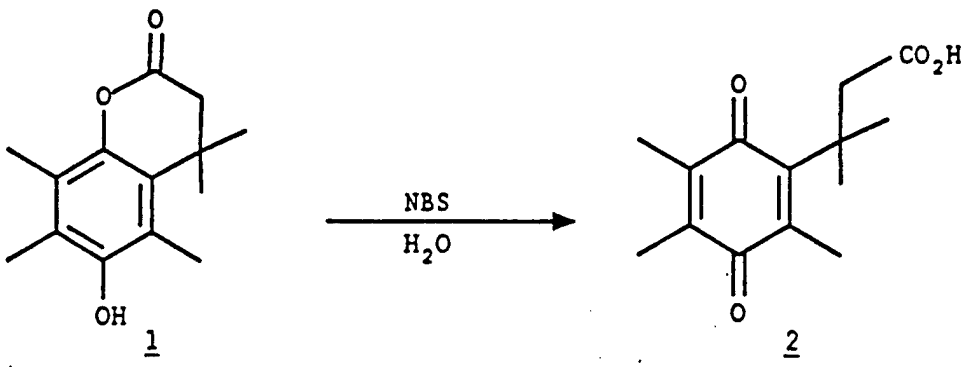
| | Eluent | Rf Value | Comment |
|---|---|---|---|
| 1. | $CH_2Cl_2$ | 0.47 | Homogeneous |
| 2. | Ether | 0.83 | Homogeneous |
| 3. | Ether-hexane (4:1) | 0.63 | Homogeneous |

Source of Materials

| | | |
|---|---|---|
| 1. | Methanesulfonic acid | Aldrich Chemical Co., Inc. |
| 2. | Trimethylhydroquinone | Aldrich Chemical Co., Inc. |
| 3. | Methyl 3,3-dimethylacrylate | Aldrich Chemical Co., Inc. |
| 4. | EtOAc | J.T. Baker Chemical Co. |
| 5. | $NaHCO_3$ | Aldrich Chemical Co., Inc. |
| 6. | Silica gel | EM Laboratories |
| 7. | $CH_2Cl_2$ | J.T. Baker Chemical Co. |
| 8. | Hexane | J.T. Baker Chemical Co. |

2. β,β,2,4,5-Pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (2)

Reaction Sequence:




1 2

Experimental

6-Hydroxy-4,4,5,7,8-pentamethylhydrocoumarin (1)

Please refer to the preceding synthesis, this report.

β,β,2,4,5-Pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (2)

To a stirred solution of 6-hydroxy-4,4,5,7,8-pentamethylhydrocoumarin (1) (30.8 g, 131 mmol) in 1500 mL of 10% aq. acetonitrile was added dropwise, at 25°C, a solution of N-bromosuccinimide (24.6 g, 138 mmol) in 300 mL of acetonitrile. The mixture was stirred for an hour at 25°C, diluted with $H_2O$ (3 L) and extracted with ether (3 x 1 L). The extracts were combined, washed with $H_2O$ (1.5 L), brine (1.5 L), dried ($MgSO_4$), then concentrated in vacuo to a yellow oil (34.1 g). The material was recrystallized from acetone-hexane to give 23.0 g of yellow solid. The material was suitable for further transformation. A portion (18 g) was again recrystallized from acetone (50 mL) and hexane (300 mL); yield 13.8 g, mp 105-106°C; literature[15] mp 101-103°C. A portion (3.3 g) will be transmitted to WRAIR (Lot No.

NJ14-153-3). The mother liquors from both crystallizations were concentrated, and the residue (13.7 g) was recrystallized from acetone (38 mL) and hexane (228 mL); yield, 11.8 g. The material was suitable for further transformation; total yield 30.6 g, 93.0%. Additional product (8.3 g) was obtained from a scouting run.

Anal.

| | C | H |
|---|---|---|
| Calc'd for $C_{14}H_{18}O_4$ | 67.18 | 7.25 |
| Found | 67.23 | 7.27 |

Spectral Data

Infrared (Nujol)

Major bands: 2950, 2830, 2730, 2630, 1875, 1685, 1625, 1575, 1415, 1390, 1360, 1315, 1265, 1225, 1205, 1140, 1115, 1085, 1020, 855, 670 $cm^{-1}$.

Ultraviolet (EtOH)

$\lambda_{max}$ 261 nm (log $\varepsilon$ 4.28).

Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 3.02 (s, 2H, C-2 $CH_2$); 2.14 (s, 3H, C-2' $CH_3$); 1.95 (d, 3H, J= 1.1 Hz, C-4' or C-5' $CH_3$); 1.92 (d, 3H, J=1.1 Hz, C-4' or C-5' $CH_3$); 1.44 (s, 6H, C-3 $CH_3$'s).

## Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - ultraviolet light.

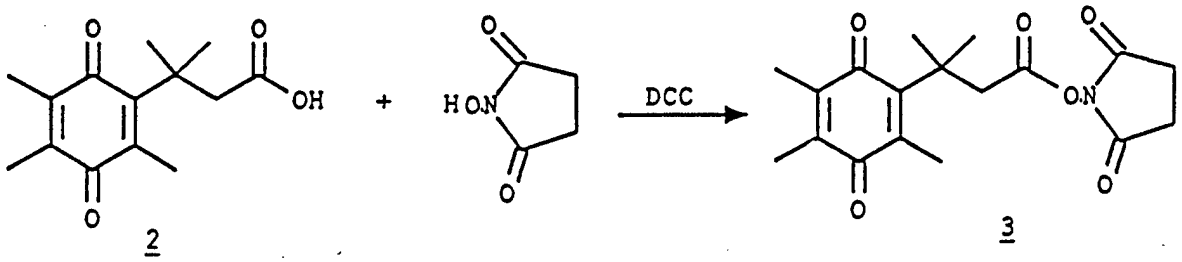
| Eluent | Rf Value | Comment |
|---|---|---|
| 1. EtOAc-hexane (2:1) | 0.51 | Homogeneous |
| 2. Ether-hexane (2:1) | 0.27 | Homogeneous |
| 3. Ether | 0.72 | Homogeneous |

## Source of Materials

| | |
|---|---|
| 1. 6-Hydroxy-4,4,5,7,8-pentamethyl-hydrocoumarin | Starks Associates, Inc. |
| 2. Acetonitrile | J.T. Baker Chemical Co. |
| 3. N-Bromosuccinimide | Aldrich Chemical Co., Inc. |
| 4. Ether | Fisher Scientific |
| 5. Acetone | J.T. Baker Chemical Co. |
| 6. Hexane | J.T. Baker Chemical Co. |

3. Succinimidyl β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoate (3)

Reaction Sequence:




2 3

Experimental[14]

β,β,2,4,5-Pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (2)

Please refer to the preceding synthesis, this report.

Succinimidyl β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoate (3)

Dicyclohexylcarbodiimide (5.0 g, 24.2 mmol) was added to a solution of quinone acid 2 (5.0 g, 20 mmol) and N-hydroxy-succinimide (2.53 g, 22 mmol) in 150 mL of dry THF at 0°C, and the mixture was stirred for 20 h at RT. The solid that separated (dicyclohexylurea) was filtered off, and the filtrate was concentrated in vacuo, diluted with EtOAc (50 mL) and again filtered. The filtrate was concentrated, and the solid residue (8.8 g) was chromatographed on a column of silica gel (500 g) using EtOAc-hexane (1:1) as the eluent.

Fractions 1-5 contained front-running impurity and were discarded. Fractions 5-10, containing product, were concentrated and the residue (6.4 g) was crystallized from EtOAc (40 mL); yield, 5.0 g. The material was suitable for further transformation. A portion (2.9 g) was recrystallized from EtOAc (20 mL) to give 2.4 g of pure material. A portion (2.0 g) will be transmitted to WRAIR (Lot No. NJ14-156-3). Additional product (31.6 g) was obtained from a larger run.

Anal.

| | | C | H | N |
|---|---|---|---|---|
| Calc'd for | $C_{18}H_{21}NO_6$ | 62.24 | 6.09 | 4.03 |
| | Found | 62.01,61.96 | 6.13,6.14 | 3.98,3.90 |

Spectral Data

Infrared (KBr)

Major bands: 1785, 1760, 1715, 1630, 1570, 1415, 1350, 1270, 1190, 1040 $cm^{-1}$.

Ultraviolet (Ethanol)

$\lambda_{max}$ 260 nm (log $\varepsilon$ 4.20).

Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 3.28 (s, 2H, $CH_2$); 2.78 (s, 4H, succinimidyl $CH_2$'s); 2.17 (s, 3H, 2-$CH_3$); 1.96 (t, 6H, J= 0.8 Hz, 4- and 5- $CH_3$'s); 1.53 (s, 6H, gem $CH_3$'s)

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - ultraviolet light.

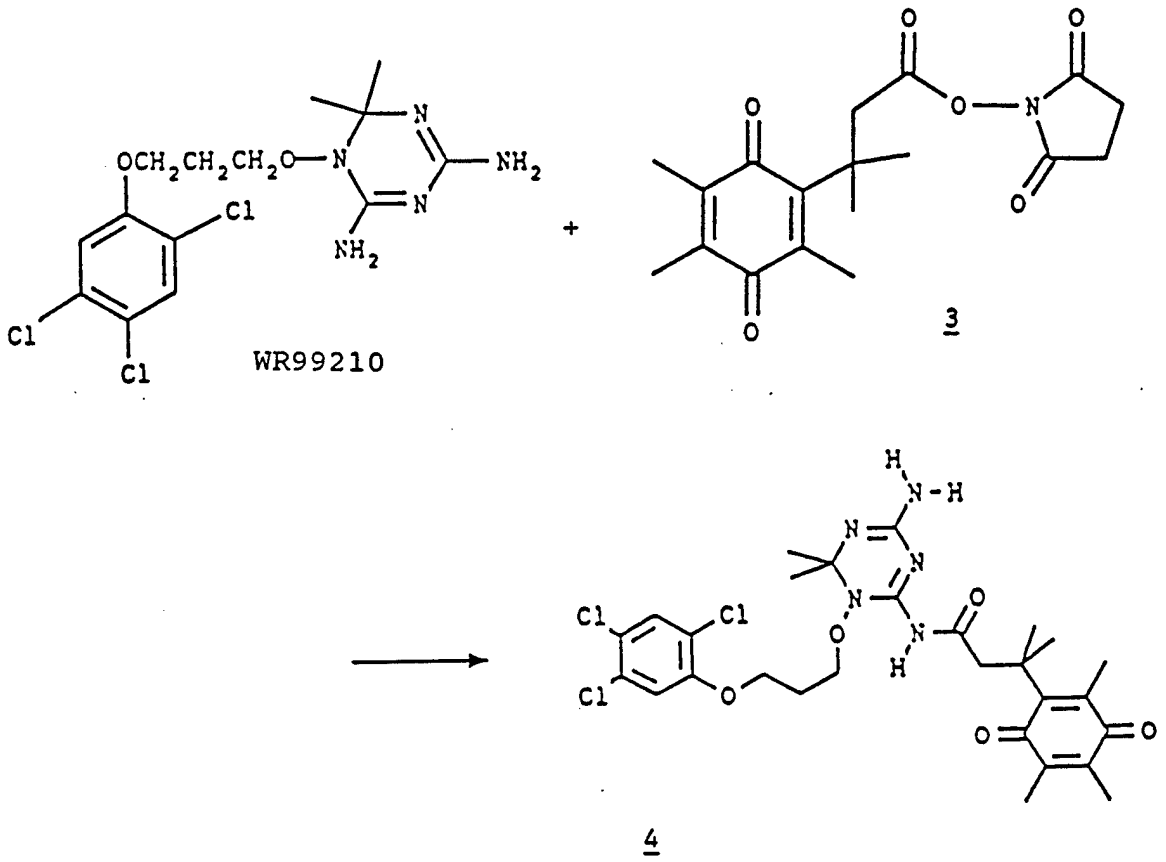
| | Eluent | Rf Value | Comment |
|---|---|---|---|
| 1. | EtOAc-hexane (2:1) | 0.74 | Homogeneous |
| 2. | Ether-hexane (2:1) | 0.20 | Homogeneous |
| 3. | Ether | 0.72 | Homogeneous |

Source of Materials

| | | |
|---|---|---|
| 1. | Dicyclohexylcarbodiimide | Aldrich Chemical Co., Inc. |
| 2. | $\beta,\beta$,2,4,5-Pentamethyl 3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid | Starks Associates, Inc. |
| 3. | N-Hydroxysuccinimide | Aldrich Chemical Co., Inc. |
| 4. | THF | Aldrich Chemical Co., Inc. |
| 5. | EtOAc | J.T. Baker Chemical Co. |
| 6. | Silica gel | EM Laboratories |
| 7. | Hexane | J.T. Baker Chemical Co. |

4. WR99210 Prodrug; amide from β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure) (4)

Reaction Sequence:



WR99210 3 4

Experimental

Succinimidyl β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoate (3)

Please refer to the preceding synthesis, this report.

WR99210 Prodrug; amide from β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure) (4)

A suspension of WR99210 (23.8 g, 0.060 mol) and 3 (21.0 g, 0.060 mol) in DMF (400 mL) was heated at 60°C for 20 h,

under an argon atmosphere. The mixture was cooled and the solvent was removed in vacuo to yield a residue (51.7 g) as a thick brown oil. The oil was purified by chromatography on a column (500 g) of $SiO_2$ using EtOAc-hexane (1:1) as the eluent. Fractions containing product were combined then concentrated to a yellow oil (21.9 g). The oil was rechromatographed on a 2 kg silica gel column using the same solvent system. The following fractions were collected: 5-16 (7.6 g), 17-23 (5.8 g) & 24-32 (1.9 g). Fractions 5-16 (7.6 g) were again rechromatographed to give 2.4 g of product. Recrystallization from 2-propanol (20 mL) gave pure product (1.7 g, 70.8% recovery), mp 170-172°C[16]. A portion (1.4 g) was transmitted to WRAIR on December 13, 1995 (Lot No. NJ12-109-3). Note that this product, lot number NJ12-109-3, was never fully characterized due to apparent instability. See discussion on page 11 of this report.

Anal.

| | C | H | N |
|---|---|---|---|
| Calc'd for $C_{28}H_{34}Cl_3N_5O_5$ | 53.64 | 5.47 | 11.17 |
| Found | 53.82 | 5.50 | 11.06 |

Spectral Data

Infrared (KBr)

$\nu$ 3296.1, 3196.5, 3073.2, 2974.6, 2924.5, 2870.1, 1707.1, 1666.1, 1639.1, 1580.2, 1466.7, 1379.6, 1329.8, 1279.9, 1240.6, 1181.8, 1136.2, 1081.2, 1059.6, 1026.8, 977.5, 938.9, 897.3, 844.9, 819.1, 745.5, 679.9, 649.8, 593.7, 414.2 $cm^{-1}$.

Ultraviolet (Ethanol)

$\lambda_{max}$ 236 nm (sh, log $\varepsilon$ 4.32); 289 nm (4.31); 295 nm (sh, 3.71).

Nuclear Magnetic Resonance ($CDCl_3$)

Spectral data (NMR) are not in full accord with the intended structure. Furthermore, the spectrum of the material has changed subsequent to shipment.

$\delta$ 10.34 (s, 1, NH); 7.42 (s, 1, ClCCHCCl); 7.25 (s, 2, $NH_2$); 7.22 (s, 1, ClC-CHCO); 4.25 (t of d, 1, J= 9.2/6.8 Hz, $\underline{CH_2}ON$); 4.17 (t of d, 1, J= 9.2/6.6 Hz, $\underline{CH_2}ON$); 4.07 (t of d, 1, J= 10.0/5.7 Hz, $\underline{CH_2}O$); 3.99 (t of d, 1, J= 10.0/5.7 Hz, $CH_2O$); 3.00 (d, 1, J= 16.8 Hz CHCO); 2.26 (d, 1, J= 16.8 Hz, CHCO); 2.18 (m, 2, $CH_2\underline{CH_2}CH_2$); 2.02 (d, 3, J= 0.9 Hz, quinone $CH_3$); 1.98 (d, 3, J= 0.9 Hz, quinone $CH_3$); 1.24 (d, 3, quinone $CH_3$); 1.17 (d, 6, 2 x $CH_3$, geminal); 1.1 (s, 3, triazine $CH_3$); 0.98 (s, 3, triazine $CH_3$).

Thin Layer Chromatography

EM precoated TLC plates, glass support, 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection-ultraviolet light.

| | Eluent | Rf Value | Comment |
|---|---|---|---|
| 1. | EtOAc-hexane (1:1) | 0.42 | Homogeneous |
| 2. | EtOH-$NH_4OH$ (20:1) | 0.86 | Homogeneous |
| 3. | $CH_2Cl_2$-$CH_3OH$ (19:1) | 0.51 | Homogeneous |

Source of Materials

| | | |
|---|---|---|
| 1. | WR99210 | Starks Associates, Inc. |
| 2. | Succinimidyl β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoate | Starks Associates, Inc. |
| 3. | DMF | Aldrich Chemical Co., Inc. |
| 4. | $SiO_2$ | EM Laboratories |
| 5. | EtOAc | J.T. Baker Chemical Co. |
| 6. | Hexane | J.T. Baker Chemical Co. |
| 7. | 2-Propanol | Aldrich Chemical Co., Inc. |

5. $N^1$-3,4-Dichlorophenyl-$N^5$-isopropyldiguanide, hydrochloride (chloroproguanil hydrochloride) (2)

The target compound 2 was prepared by the following sequence of reactions.

Reaction Sequence:

a. $NaN(CN)_2$ + $Cl^- NH_3^+$(isopropyl) $\xrightarrow{HCl}$ 1

b. 1 + 3,4-dichlorophenylammonium chloride $\longrightarrow$ 2

Isopropyl dicyandiamide (1)

Isopropylamine (100 mL, 1.17 mol) was added over a ten minute period to a solution of 10.2N hydrochloric acid (107.8 mL, 1.10 mol) and 1-butanol (1370 mL). The mixture was stirred and heated, then the water removed by distillation, at atmospheric pressure, through a Claisen head until the head temperature reached 116°C (950 mL). Additional 1-butanol (320 mL) and sodium dicyanamide (100 g, 96% purity, 1.08 mol) was added to the solution at room temperature. The reaction mixture was refluxed for 2.75 h, cooled to room temperature, then filtered. The insolubles (NaCl) were washed with 1-butanol (2 x 50 mL) then discarded. The filtrate, together with the washings, was concentrated in vacuo at 40°C to a

clear viscous oil (154 g). The oil was dissolved in dioxane (970 mL at 55°C). After cooling to room temperature, the white solid, that separated was collected, then dried (room temperature 4-5 h). NMR showed the material contained ca. 0.5 molar eq. of dioxane, 169 g (92%). Additional product of similar purity (428.6 g) was obtained from other runs.

Spectral Data

Infrared (Nujol)

Major bands: 3360, 3180, 3140, 2960, 2910, 2850, 2160(CN), 1670, 1620, 1540, 1470, 1455, 1425, 1380, 1370, 1290, 1260, 1170, 1130, 1110, 990, 980, 860 $cm^{-1}$.

Nuclear Magnetic Resonance (DMSO-$d_6$)

$\delta$ 6.75 (broad s, 1, NH); 6.55 (broad s, 2, 2xNH); 3.75 [m, 1, C<u>H</u>$(CH_3)_2$]; 3.40 (s, dioxane); 1.10 (d, 6H, 2 x $CH_3$).

$N^1$-3,4-Dichlorophenyl-$N^5$-isopropyldiguanide, hydrochloride (chloroproguanil hydrochloride) (2)

A solution of 3,4-dichloroaniline (159.2 g, 0.9826 mol), isopropyldicyandiamide hemisolvate (1) (168.9 g, 0.9922 mol of material containing 0.5 molar eq. of dioxane), 10.2N hydrochloric acid (97.3 mol, 0.9925 mol) and 2-ethoxyethanol (1.1 L) was refluxed for 70 min, and cooled to room temperature overnight. Ethyl acetate (1690 mL) was added dropwise to the stirred suspension over 2.5 h. After storing at room temperature for 4 h, the mixture was filtered, and the white crystals were washed with ethyl acetate (2 x 200 mL) then dried in vacuo at room temperature; yield 189.2 g (59%), m.p. 248-250°C; literature[17] mp 246°C. Additional product of similar purity (496.6 g), mp 247-247.5°C (d) was prepared in a similar way. A portion (29.1 g) was transmitted to WRAIR on January 5, 1996 (Lot No. NJ19-28-1).

Anal.

| | C | H | Cl | N |
|---|---|---|---|---|
| Calc'd for $C_{11}H_{16}Cl_3N_5$ | 40.69 | 4.97 | 32.76 | 21.57 |
| Found | 40.43 | 4.85 | 32.80 | 21.37 |
| | 40.42 | 4.88 | 21.30 | |

Spectral Data

Infrared (Nujol)

Major bands: 3280, 3080, 2920, 2860, 1630, 1600, 1565, 1530, 1470, 1410, 1365, 1290, 1250, 1230, 1170, 1130, 1030, 870, 810 $cm^{-1}$.

Ultraviolet (MeOH)

$\lambda_{max}$ 260 nm (log $\varepsilon$ 4.37).

Nuclear Magnetic Resonance (DMSO-$d_6$)

$\delta$ 10.39 & 10.12 (2 broad s, 1H, $^{\oplus}$NH); 8.1-8.0 (m, 1H, NH); 7.84 (d, 2, J= 2.5 Hz, H-5, H-6); 7.58 (d, 1, J= 8.7 Hz, H-2); 7.38 (s, 1 NH); 7.28 (s, 1, NH); 6.89 (s, 1, NH); 3.76 (m, 1, $\underline{CH}(CH_3)_2$); 1.18 (d, 6, $CH(\underline{CH_3})_2$.

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - ultraviolet light.

| Eluent | Rf Value | Comment |
|---|---|---|
| MeOH-$NH_4OH$ (19:1) | 0.16 | Homogeneous |

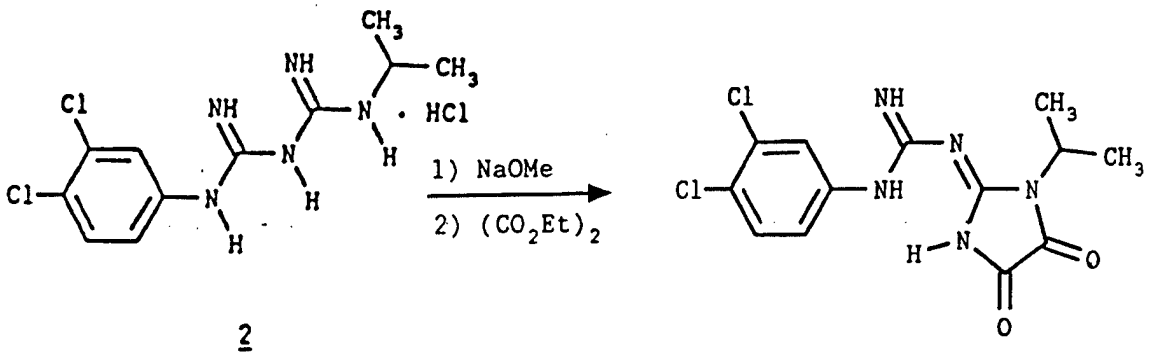
Source of Materials

| | | |
|---|---|---|
| 1. | Isopropylamine | Aldrich Chemical Co., Inc. |
| 2. | HCl | J.T. Baker Chemical Co. |
| 3. | $NaN(CN)_2$ | Aldrich Chemical Co., Inc. |
| 4. | Dioxane | Aldrich Chemical Co., Inc. |
| 5. | 3,4-Dichloroaniline | Aldrich Chemical Co., Inc. |
| 6. | Isopropyldicyandiamide | Starks Associates, Inc. |
| 7. | 2-Ethoxyethanol | Aldrich Chemical Co., Inc. |
| 8. | Ethyl acetate | EM Science Co. |

6. 1-(3,4-Dichlorophenyl)-3-(1-isopropyl-4,5-dioxo-2-imidazolidinylidene)guanidine (3)

The target compound 3 was prepared by the following reaction.

Reaction Sequence:




2 3

Experimental

$N^1$-3,4-Dichlorophenyl-$N^5$-isopropyldiguanide, hydrochloride (chloroproguanil hydrochloride) (2)

Please refer to the preceding synthesis, this report.

1-(3,4-Dichlorophenyl)-3-(1-isopropyl-4,5-dioxo-2-imidazolidinylidene)guanidine (3)

A suspension of $N^1$-3,4-dichlorophenyl-$N^5$-isopropyldiguanide hydrochloride (2) (101.0 g, 0.311 mol), sodium methoxide (80.6 mL of 25 wt % solution in methanol, 0.35 mol) and absolute methanol (1050 mL) was brought to reflux, cooled to room temperature (20°C), then filtered. The insoluble solid was washed with absolute methanol (50 mL). The washings were combined with the filtrate, and to this solution was

added in one portion diethyl oxalate (42 mL, 0.311 mol). The suspension was stirred at 20°C for 1.5 h then filtered. The yellow solid was washed with methanol (4 x 60 mL) then dried in vacuo at room temperature to constant weight; 75.2 g (71%). The material was combined with 85 g of product of similar purity to give 160.2 g, mp 243-244°C(d); literature[17] mp 248°C(d), 250-251°C(d) & 253-254°C(d). A portion (156.6 g) was transmitted to WRAIR on January 29, 1996 (Lot No. NJ19-54-1). Additional material (193.3 g), mp 245-246°C was obtained from another reaction. A portion (192.3 g) was transmitted to WRAIR on January 29, 1996 (Lot No. NJ19-40-1).

Anal.

| | C | H | Cl | N |
|---|---|---|---|---|
| Calc'd for $C_{13}H_{13}Cl_2N_5O_2$ | 45.63 | 3.83 | 20.72 | 20.47 |
| Found (Lot No. NJ19-54-1) | 45.70 | 3.84 | 20.81 | 20.45 |
| (Lot No. NJ19-40-1) | 45.70 | 3.83 | 20.55 | 20.63 |

Spectral Data

Infrared (Nujol)

The infrared spectra for both lots were identical to the spectrum of a reference sample provided to us by COL J. Scovill.

Major bands: 3320, 3220, 3120, 2900, 2840, 1760, 1730, 1650, 1600, 1470(b), 1370, 1320, 1310, 1255, 1220, 1180, 1130, 1105, 1070, 1025, 990, 940, 860, 800, 750 $cm^{-1}$.

Ultraviolet (Methanol)

| | |
|---|---|
| Lot No. NJ19-40-1 | $\lambda_{max}$ 237 nm (log $\varepsilon$ 4.19); 269 mn (4.27). |
| Lot No. NJ19-54-1 | $\lambda_{max}$ 237 nm (log $\varepsilon$ 4.17); 270 mn (4.25). |

Nuclear Magnetic Resonance (DMSO-$d_6$ & DCl)

The NMR spectra for both lots were identical to the spectrum of a reference sample provided to us by COL J. Scovill. All spectra show the material to be a mixture of several isomers.

$\delta$ 7.9-7.2 (m, 6H); 4.3-3.5 (m, 1H); 1.3 - 1.0 (m, 6H).

Thin Layer Chromatography

A satisfactory TLC system was not found because of the limited solubility of the material in most suitable solvents.

Source of Materials

| | |
|---|---|
| 1. $N^1$-3,4-Dichlorophenyl-$N^5$-isopropyldiguanide, HCl | Starks Associates, Inc. |
| 2. Sodium methoxide (25% wt. solution in $CH_3OH$) | Aldrich Chemical Co., Inc. |
| 3. Diethyl oxalate | Aldrich Chemical Co., Inc. |
| 4. $CH_3OH$ | J.T. Baker Chemical Co. |

7. 6-Cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (6)

The intermediate (6) was prepared by the following sequence of reactions:

Reaction Sequence:

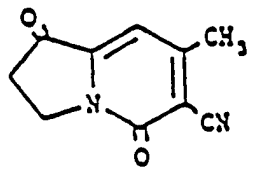
a. $EtOCCOEt + H_3CCCH_3 \xrightarrow{NaOEt} H_3CCCH_2CCOEt$

1

b. 1 + $CH(OEt)_3$ → $H_3CCCH{=}C(OEt)COEt$

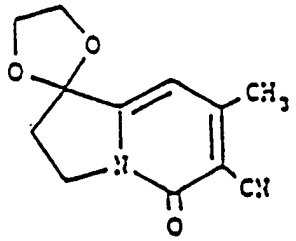
2

c. 2 → 1. $H_2NCCH_2CN$; 2. $H_2C{=}CHCO_2Me$; 3. HCl/HOAc



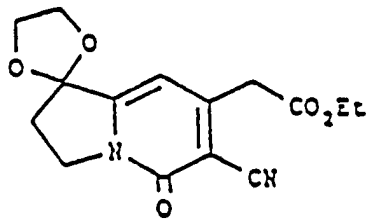
3

d. 3 + $HOCH_2CH_2OH$ →

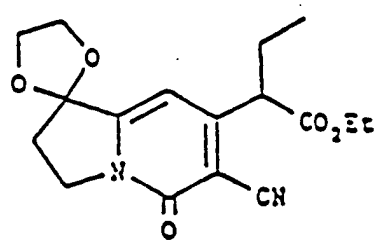


4

e. 4 + $EtOCOEt \xrightarrow{KH}$



5

f. 5 + EtI $\xrightarrow{+\ OK}$ 

6

## Experimental

The material was prepared as discribed on pp. 56-69, this report. A portion (5.3 g) was transmitted to WRAIR on December 21, 1996 (Lot No. NJ12-124-1).

Anal.

| | | C | H | N |
|---|---|---|---|---|
| Calc'd for | $C_{17}H_{20}N_2O_5$ | 61.44 | 6.07 | 8.43 |
| | Found | 61.51 | 6.07 | 8.42 |

Spectral Data

Infrared (Nujol)

Major bands: 2920, 2840, 2220, 1730, 1660, 1600, 1530, 1460, 1370, 1340, 1320, 1260, 1230, 1190, 1090, 1020, 930, 810, 770 $cm^{-1}$.

Ultraviolet (EtOH)

$\lambda_{max}$ 216 nm (log $\varepsilon$ 4.28); 335 nm (4.02); 340 nm (sh, 4.01).

Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 6.40 (s, 1, H at C-8); 4.23-4.11 (m, 8, $OCH_2CH_2O$, $CH_3\underline{CH_2}CO$, $CH_2N$); 3.93 (t, 1, J= 7.60, $\underline{CH}CH_2CH_3$); 2.41 (t, 2, J= 6.90, $CH\underline{CH_2}CH_3$); 2.12 (m, 1, $NCH_2\underline{CH_2}$); 1.78 (m, 1, $NCH_2\underline{CH_2}$); 1.26 (t, 3, J= 7.10, $CH_3$); 0.97 (t,

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; visulization - UV.

| | Eluent | Rf Value | Comment |
|---|---|---|---|
| 1. | EtOAc-hexane (4:1) | 0.32 | homogeneous |
| 2. | $CH_2Cl_2$-EtOAc (7:3) | 0.48 | homogeneous |
| 3. | $CH_2Cl_2$-acetone (4:1) | 0.70 | homogeneous |

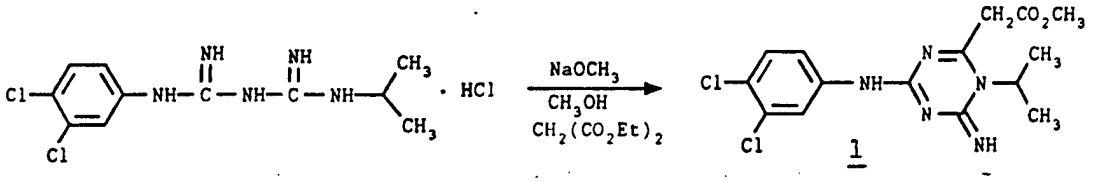
Source of Material

Please refer to pp. 67-69, this report.

8. 1,3,5-Triazine-2-acetic acid, 4-[(3,4-dichlorophenyl)amino]-1,6-dihydro-6-imino-1-(1-methylethyl)-, methyl ester (1)

The target compound (tentative structure) was prepared by the following reaction sequence.

Reaction Sequence




Experimental

1,3,5-Triazine-2-acetic acid, 4-[(3,4-dichlorophenyl)amino]-1,6-dihydro-6-imino-1-(1-methylethyl)-, methyl ester (1)

To a solution of $N^1$-3,4-dichlorophenyl-$N^5$-isopropyl-diguanide hydrochloride (chloroproguanil hydrochloride) (1.00 g, 3.10 mmol) in methanol (10.0 mL) was added sodium methoxide in methanol (25 wt %, 0.8 mL, 3.50 mmol). The suspension was heated to reflux then cooled to 20°C and filtered. To the filtrate was added diethyl malonate (468 μL, 3.10 mmol) and the mixture was heated at reflux for 20 h. After cooling to 20°C, the reaction mixture was diluted with EtOAc (150 mL) and washed with 0.1N HCl (2 x 100 mL) and $H_2O$ (100 mL), then dried ($MgSO_4$), filtered, and concentrated to give the crude product (0.4 g), which was purified by silica gel chromatography (1:1 EtOAc:hexanes, 40 g $SiO_2$) to give the triazine (tentative structure) 1 (230 mg 21%) as a pale yellow solid; mp 108-110°C. A portion (150 mg) was transmitted to WRAIR on May 22, 1996 (Lot No. NJ20-76-2).

Anal.

| | | C | H | N | Cl |
|---|---|---|---|---|---|
| Calc'd for | $C_{15}H_{17}Cl_2N_5O_2$ | 48.66 | 4.63 | 18.92 | 19.15 |
| | Found | 48.84 | 4.67 | 18.91 | 19.06 |

Spectral Data

Infrared (KBr)

Major bands: 3409, 3356, 2955, 2926, 2328, 2304, 1737, 1612, 1569, 1508, 1477, 1443, 1405, 1323, 1299, 1199, 1173, 1125, 994, 815, 497 $cm^{-1}$.

Nuclear Magnetic Resonance ($CDCl_3$)

(mixture of tautomers)

$\delta$ 8.07 and 7.87 (2 br s, 1H, C=N<u>H</u>); 7.34-6.97 (m, 3H, Ar); 5.23 and 5.16 (2 br s, 1H, NH); 4.20-4.08 (m, 1H, <u>CH</u>$(CH_3)_2$); 3.75 (s, 3H, $CO_2$<u>$CH_3$</u>); 3.63 and 3.56 (2 s, 2 H, $CH_2$); 1.25 and 1.22 (2 d, 6H, CH(<u>$CH_3$</u>$)_2$).

Mass Spectrum

Method of Ionization = Electrospray (positive)

Calc'd for $C_{15}H_{17}Cl_2N_5O_5$ = 369.1

Found: 370.1 $(m + H)^+$.

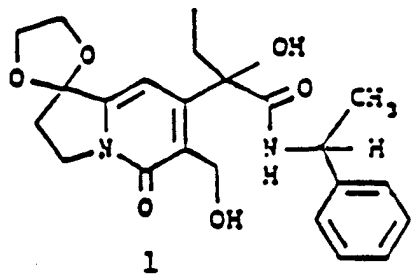
Source of Materials:

| | | |
|---|---|---|
| 1. | Chloroproguanil hydrochloride | Starks Associates, Inc. |
| 2. | Methanol | J.T. Baker Chemical Co. |
| 3. | Sodium methoxide (25 wt % in $CH_3OH$) | Aldrich Chemical Co., Inc. |
| 4. | Diethyl malonate | Aldrich Chemical Co., Inc. |
| 5. | Ethyl acetate | E.M. Science |
| 6. | Hydrochloric acid | J.T. Baker Chemical Co. |
| 7. | Magnesium sulfate | J.T. Baker Chemical Co. |
| 8. | Silica gel | E.M. Science |
| 9. | Hexanes | J.T. Baker Chemical Co. |

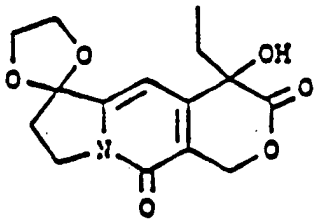
9. (*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano-[3,4-*f*]-indolizine-3,6,10-trione (5)

The target compound 5 was prepared by the following sequence of reactions.

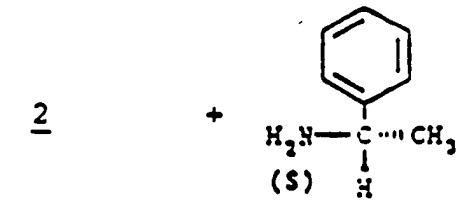
Reaction Sequence:

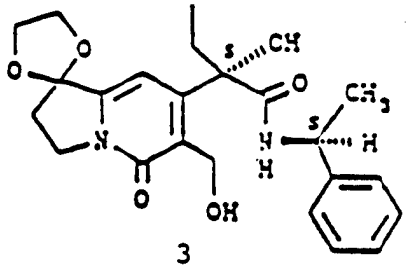


1



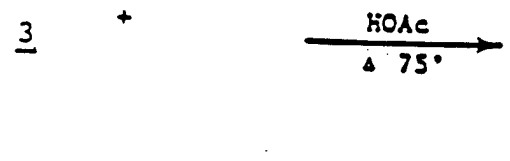
2

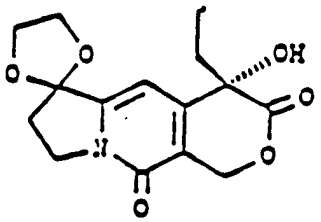




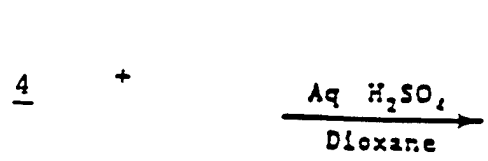
3

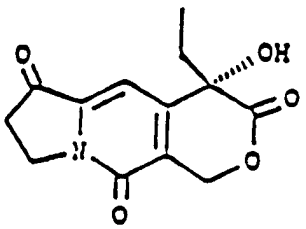
and (*R,S*)-Diastereomer 3a





4





5

## Experimental

### (*R*,*S*)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3-10-(6*H*)-dione (2)

A solution of crude (*R*,*S*)-2-hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(*R*,*S*)-α-methylbenzyl]butyramide (1) (71.0 g, 0.16 mol) and AcOH (1.3 L) was stirred at 75°C for 3 h. The solvent was removed in vacuo and the last traces of AcOH were removed by codistillation with toluene (2 x 50 mL). The residue (107.7 g) was chromatographed on silica gel (1 kg) using $CH_2Cl_2$-acetone (4:1) as the eluent. Fractions containing product were combined and concentrated in vacuo to obtain 43.9 g of product suitable for further transformation.

### (*S*)-2-Hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(*S*)-(-)-α-methylbenzyl]butyramide (3)

A solution of racemic 4-ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3,10-(6*H*)-dione (2) (14.2 g, 46.2 mmol) and (S)-(-)-α-methylbenzylamine (45.0 g, 0.372 mol), maintained under an argon atmosphere, was heated at 70°C for 20 h then the excess amine was removed by vacuum distillation. The oily residue (25.5 g) was suspended in toluene (150 mL), and the mixture was stirred for 2 h. The resulting solid was collected on a filter, washed with toluene (2 x 25 mL), hexane (2 x 25 mL), then dried; yield 9.3 g. This was slurried in $CH_2Cl_2$ (80 mL). The light suspension was filtered, and toluene (150 mL) was added to the filtrate. The mixture was stirred for 2 h. The precipitated solid was collected, washed with hexane (50 mL) and dried in vacuo to give 7.0 g (35.4%) of 3 as a white solid. Additional product (18.8 g) was obtained from a larger reaction. The material was suitable for further transformation.

(*S*)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-f]indolizine-3,10-(6H)-dione (4)

A solution of (*S*)-2-hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(*S*)-(-)-α-methylbenzyl]butyramide (3) (4.7 g, 10.97 mmol) and AcOH (100 mL) was stirred at 75°C for 2 h then the solvent was removed by evaporation. The residue was dissolved in EtOAc then chromatographed on silica gel (200 g) eluted with EtOAc-MeOH (10:1) to give 3.1 g (92%) of 4; m.p. 166-168°C $[\alpha]_D^{22}$ = +100.32° (C= 1.00 in $CHCl_3$). An additional reaction was carried out to give a total of 12.1 g of (4) via this route.

(*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-f]-indolizine-3,6,10-trione (5)

A solution of (*S*)-4-ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3,10-(6*H*)-dione (4) (15.0 g, 48.8 mmol), $H_2SO_4$ (260 g), water (220 mL), and dioxane (390 mL) was heated at reflux for 80 min, cooled, then extracted with $CH_2Cl_2$ (3 x 500 mL). The combined organic layers were washed with water (2 x 200 mL). The aqueous layer was neutralized to pH6 with solid $Na_2CO_3$ and re-extracted with $CH_2Cl_2$ (2 x 200 mL). The combined organic layers were dried over $MgSO_4$ then concentrated in vacuo to a residue. The residue was dissolved in MeOH (200 mL) and charcoaled with carbon (1 g). The solvent was removed, and the residue was dissolved in $CH_2Cl_2$ (100 mL). The solid was precipitated by dropwise addition of hexane (100 mL). This was collected on a filter and dried in vacuo at 40°C to 10.3 g of 4; mp 170-172°C. Literature[13] mp 174-176°C, $[\alpha]_D^{20}$ = 105.6° (C= 0.765; $CHCl_3$/MeOH 4:1); literature[13] $[\alpha]_D^{20}$ = +105.2° (C= 0.49; $CHCl_3$/MeOH 4:1). A portion (10.2 g) was transmitted to WRAIR on June 19, 1996 (Lot No. NJ19-72-4).

Anal.

| | | C | H | N |
|---|---|---|---|---|
| Calc'd for | $C_{13}H_{13}NO_5$ | 58.79 | 5.03 | 5.27 |
| | $\cdot$ 0.13 $H_2O$ | | | |
| | Found | 58.76 | 5.01 | 5.25 |

Spectral Data

Infrared (Nujol)

Major bands: 3400, 2900, 2840, 1740, 1660, 1610, 1455, 1385, 1345, 1315, 1260, 1230, 1200, 1150, 1100, 1055, 995 $cm^{-1}$.

Ultraviolet (EtOH)

$\lambda_{max}$ 228 nm (sh, log $\varepsilon$ 4.03); 260 nm (sh, 3.39); 337 nm (3.88).

Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 7.22 (s, 1, pyridone H); 5.66 (d, 1, J= 17.0 Hz, H at C-1); 5.24 (d, 1, J= 17.0 Hz, H at C-1); 4.39 (t, 2, $COCH_2$); 3.76 (s, 1, OH); 2.96 (t, 2, $NCH_2$); 1.81 (q, 2, $\underline{CH_2}$-$CH_3$); 0.98 (t, 3, $CH_3$).

Source of Material

| | | |
|---|---|---|
| 1. | (*R,S*)-2-Hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(*R,S*)-$\alpha$-methylbenzyl]-butyramide | Starks Associates, Inc. |
| 2. | AcOH | J.T. Baker Chemical Co. |
| 3. | Toluene | J.T. Baker Chemical Co. |

| | | |
|---|---|---|
| 4. | Silica gel | EM Laboratories |
| 5. | $CH_2Cl_2$ | J.T. Baker Chemical Co. |
| 6. | Acetone | J.T. Baker Chemical Co. |
| 7. | (*R,S*)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]-indolizine-3,10-(6*H*)-dione | Starks Associates, Inc. |
| 8. | (*S*)-(-)-α-Methylbenzylamine | Aldrich Chemical Co., Inc. |
| 9. | Hexane | J.T. Baker Chemical Co. |
| 10. | (*S*)-2-Hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(*S*)-α-methylbenzyl]-butyramide | Starks Associates, Inc. |
| 11. | EtOAc | J.T. Baker Chemical Co. |
| 12. | $CH_3OH$ | J.T. Baker Chemical Co. |
| 13. | (*S*)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]-indolizine-3,10-(6*H*)-dione | Starks Associates, Inc. |
| 14. | $H_2SO_4$ | J.T. Baker Chemical Co. |
| 15. | Dioxane | J.T. Baker Chemical Co. |
| 16. | $Na_2CO_3$ | Aldrich Chemical Co., Inc. |

10. (*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano-[3,4-*f*]-indolizine-3,6,10-trione (12)

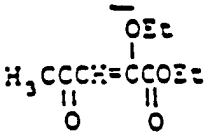
The target compound 12 was prepared by the following sequence of reactions.

Reaction Sequence:

a. $EtOCCOEt + H_3CCCH_3 \xrightarrow{NaOEt} H_3CCCH_2CCOEt$

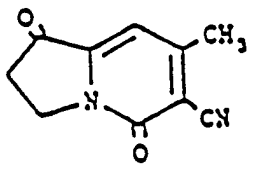
1

b. 1 + $CH(OEt)_3$ ⟶



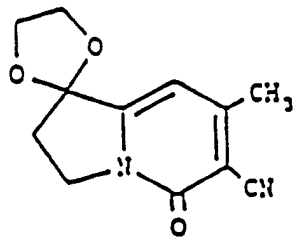
2

c. 2 → 1. $H_2NCCH_2CN$; 2. $H_2C{=}CHCO_2Me$; 3. HCl/HOAc



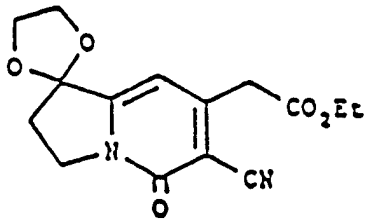
3

d. 3 + $HOCH_2CH_2OH$ ⟶



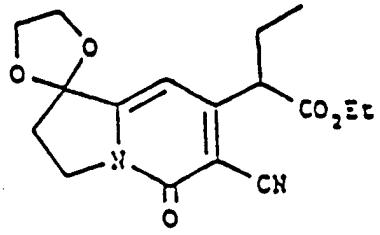
4

e. 4 + EtOCOEt $\xrightarrow{KH}$



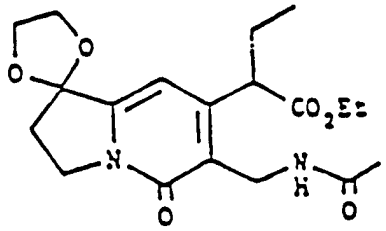
5

f. 5 + EtI $\xrightarrow{+\ OK}$



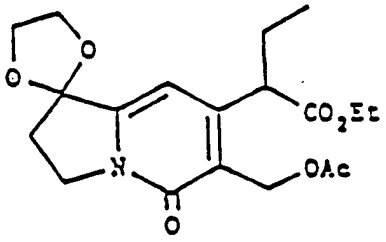
6

g. 6 + $H_2$ $\xrightarrow[\text{Nickel}\ \text{HOAc}]{\text{Raney}}$



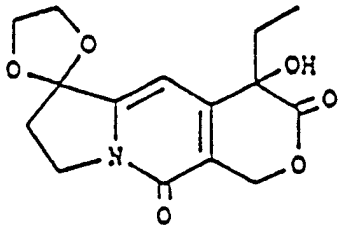
7

h. 7 + $NaNO_2$ $\xrightarrow{HOAc}$

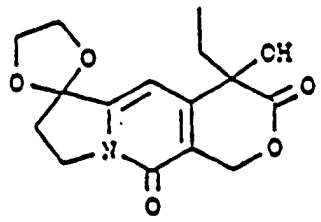
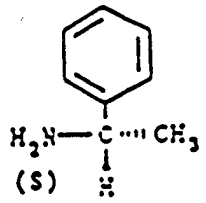
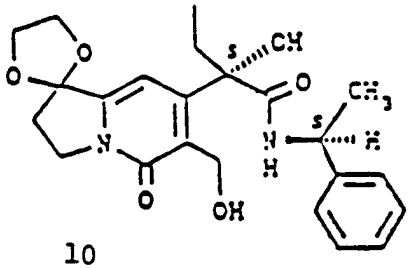


8

i. 8 + $O_2$ $\xrightarrow{K_2CO_3}$

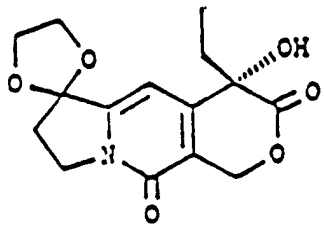


9

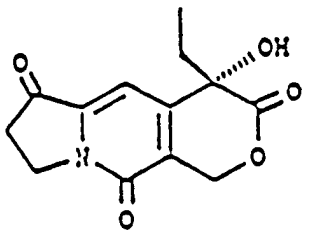
j.  +  $\xrightarrow[70°C]{\Delta}$ 

9 — 10

and (R,S)-Diastereomer 10a

k. 10 + $\xrightarrow[\Delta\ 75°]{HOAc}$ 

11

i. 11 + $\xrightarrow[Dioxane]{Aq\ H_2SO_4}$ 

12

## Experimental

### Ethyl acetopyruvate (1)

To a cold (0°C), stirred solution (under argon) of ethanolic NaOEt (5000 g of 21% in EtOH, 15.44 mol) in EtOH (6 L) was added a mixture of diethyl oxalate (2052 g; 14.04 mol) and acetone (814 g; 14.04 mol) during 1 h. The temperature was maintained between 0-5°C during the addition. The resulting suspension was stirred at ambient temperature for 16 h then separated by filtration. The collected solid was washed with $Et_2O$ (2 x 2 L) then dried in vacuo at 40°C to give 2182.8 g (86.4%) of compound 1 as the sodium salt. This material was suspended (partial solution) in cold (0°C) $H_2O$ then acidified with concentrated $H_2SO_4$. The resulting mixture was extracted with $CH_2Cl_2$ (2 x 10 L). The organic extracts were combined, washed with 5% aqueous $NaHCO_3$ (4 L), dried over $Na_2SO_4$ then concentrated in vacuo to a brown oil. This oil was vacuum distilled to give 1157.8 g (52.2%) of compound 1; bp 95-105°C at 15 mm. The material was suitable for further transformation.

### Ethyl 2-ethoxy-4-oxopent-2-enoate (2)

A mixture of ethyl acetopyruvate (1) (1157.8 g, 7.32 mol), triethyl orthoformate (1191.5 g, 8.04 mol), ammonium chloride (79.2 g, 1.48 mol) and ethanol (2.1 L) was stirred at RT for 120 h then concentrated in vacuo to ~2 L. The solid was filtered off then washed with EtOH (250 mL). The filtrate and washings were combined then concentrated in vacuo to an oil. The oil was dissolved in ether (3 L), and the solution was washed with 2N $K_2CO_3$ (3 x 1 L), dried ($Na_2SO_4$), then concentrated (1357.6 g). The material was distilled in vacuo to yield 1225.8 g (89.9%) of material 2 as pale yellow oil, bp 110-115°/10 mm. The product was suitable for further transformation.

6-Cyano-1,5-dioxo-7-methyl-1,2,3,5-tetrahydroindolizine (3)

To a warm (45°C), stirred suspension of cyanoacetamide (142.9 g; 1.70 mol), anhydrous $K_2CO_3$ (230.5 g; 1.67 mol), and dry DMF (3 L) under argon was added ethyl 2-ethoxy-4-oxo-pent-2-enoate (2) (294 g; 1.58 mol) in a thin stream during 45 min. The resulting deep red suspension was stirred at 45°C for 65 h after which time TLC (toluene:dioxane:HOAc 90:25:4) analysis indicated the starting material had been consumed. The warm (45°C) solution was diluted with $H_2O$ (57 mL) then methyl acrylate (990 mL, 947 g, 11 mol) was added in a thin stream during 45 min. The resulting red suspension was stirred at 45°C for 96 hours, cooled to room temperature then separated by filtration. The solid was washed on the funnel with DMF (2 x 250 mL) and acetone (3 x 250 mL), then suspended in $H_2O$ (3 L). This suspension (partial solution) was acidified (pH~1) with conc. HCl. The suspended solid was collected on a funnel then washed with $H_2O$ (2 x 250 mL). The wet cake (901 g) of the indolizine was dissolved in concentrated HCl (1.5 L) and glacial HOAc (1.5 L) then stirred at reflux for 18 h. TLC analysis ($CHCl_3$/acetone/MeOH, 15:4:1) showed completeness of reaction. The reaction mixture was cooled to room temperature then extensively extracted with $CH_2Cl_2$ (7 x 4 L). The organic extracts were washed with brine (16 L), dried over $MgSO_4$, then concentrated in vacuo to a residue. This material was triturated with hexane to give 95.1 g (32% overall yield from compound (2)) of the bicyclic ketone intermediate 3. Additional product (271.9 g) was obtained from other reactions. The material was suitable for further transformation.

6-Cyano-1,1-(ethylenedioxy)-7-methyl-5-oxo-1,2,3,5-tetrahydroindolizine (4)

A stirred solution (containing minor amount of insolubles) of 6-cyano-1,5-dioxo-7-methyl-1,2,3,5-tetrahydroindolizine (3) (186.5 g; 0.99 mol) in $CH_2Cl_2$ (8 L) was warmed to 30°C then 17.5 g of charcoal was added. This suspension was stirred for 0.5 h then clarified by filtration. The temperature of the filtrate was adjusted to 25°C then ethylene glycol (134.7 g; 2.17 mol) and chlorotrimethylsilane (558 mL; 4.40 mol) were added in one portion. The reaction mixture was stirred at 25°C for 7 days. TLC analysis ($CHCl_3$/acetone/MeOH 15:4:1) indicated completeness of reaction. The reaction mixture was clarified by filtration, washed with 5% NaOH (5 x 3.5 L), dried over $MgSO_4$, then concentrated in vacuo to a residue. This residue was recrystallized from MeOH (5 L) to give 121.3 g (52.7%) of purified intermediate 4. Additional product (165.5 g) was obtained from another reaction. The material was suitable for further transformation.

6-Cyano-7-[(ethoxycarbonyl)methyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (5)

A stirred suspension of 6-cyano-1,1-(ethylenedioxy)-7-methyl-5-oxo-1,2,3,5-tetrahydroindolizine (4) (111.0 g; 0.478 mol), NaH (60% min oil dispersion, 47.8 g; 1.194 mol) and anhydrous toluene (1000 mL) was heated under argon at reflux for 15 min, then a mixture of diethyl carbonate (148.7 g; 1.259 mol) and absolute EtOH (22.2 g; 28.3 mL; 0.482 mol) was added dropwise during 45 min. The reaction mixture (green suspension) was heated at reflux for 3 h then cooled to room temperature. The solid was collected on a funnel then washed on the funnel with toluene (3 x 200 mL). The solid was placed in a flask and glacial acetic acid (2 L) was slowly added

under a strong argon purge (some NaH decomposition is occurring in this step). The resulting mixture (mostly dissolved) was diluted with $H_2O$ (1388 mL) then extracted with $CH_2Cl_2$ (3 x 2 L). The combined extracts were washed with brine (2 x 1500 L), dried over $Na_2SO_4$, then concentrated in vacuo to a green solid (147.5 g). This material was combined with product of similar purity (256.6 g) then chromatographed on a 22 kg column of silica gel eluted with $CH_2Cl_2$-$CH_3OH$ (10:1) to give 309.4 g of purified product. The material was suitable for further transformation.

6-Cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (6)

A stirred suspension of 6-cyano-7-[(ethoxycarbonyl)-methyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (5) (150.0 g, 0.493 mol) in dry 1,2-dimethoxyethane (2600 mL) under argon was cooled to -65°C, then potassium *t*-butoxide (52.6 g, 0.473 mol) was added in one portion. The reaction mixture was allowed to warm to 0°C then re-cooled to -65°C. Ethyl iodide (397.4 g, 2.55 mol) was added, and the reaction mixture was stirred at -65°C for 3 h, then at room temperature for 18 h. Quenching of the mixture was carried out by the slow addition of $H_2O$ (900 mL). The resulting mixture was extracted with $CH_2Cl_2$ (3 x 1880 mL). The combined extracts were washed with brine (2 x 2000 mL), dried over $Na_2SO_4$, then concentrated in vacuo to a solid residue (168.0 g). Additional crude product (178.6 g) was obtained from another reaction. The lots were combined, then chromatographed on an 11 kg silica gel column eluted with EtOAc/hexanes (4:1), to give 272.8 g (80.4% overall) of intermediate 6. The material was suitable for further transformation.

6-(Acetamidomethyl)-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (7)

A stirred suspension of 6-cyano-7-[1'-(ethoxycarbonyl)-propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (6) (80.0 g; 0.24 mol), activated Raney nickel (47 mL of a slurry that had been washed with HOAc decantations (6x100 mL) and acetic anhydride/HOAc decantations (3:1, 6x100 mL)), and acetic anhydride/HOAc (3:1, 810 mL) was hydrogenated at 45°C and 70 psi hydrogen for 3.5 h. TLC analysis ($CHCl_3$/acetone/MeOH 15:4:1) indicated complete loss of the starting material. The catalyst was removed by filtration, and the filtrate was used directly in the next step without further isolation. Additional 6 (185.6 g) was reacted in a similar manner.

6-Acetoxymethyl-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (8)

The solution of 6-(acetamidomethyl)-7-[1'-(ethoxy-carbonyl)propyl]-1,1-(ethylenedioxy)-7-[1'-(ethoxycarbonyl)-propyl]-5-oxo-1,2,3,5-tetrahydroindolizine (7) (90.5 g; 0.24 mol) from the previous step was cooled to 0°C, under argon, with magnetic stirring. Sodium nitrite (90.0 g, 1.3 mol) was added portionwise, during a 30 min period, while maintaining the internal temperature at 0-5°C. After complete addition, the reaction mixture was stirred at ambient temperature for 20 h, then concentrated in vacuo. The resulting residue was suspended in $CCl_4$ (1 L), heated at reflux for 3 h, then cooled to ambient temperature. The solution was washed with $H_2O$ (3x1 L), dried over $Na_2SO_4$, then concentrated in vacuo to give 68.4 g of intermediate 8 as a brown oil. This crude material (95.7 g) was used directly in the next step without further purification or characterization. Additional 8 (241.2 g) was obtained from other reactions.

(*R,S*)-4-Ethyl-6-6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3,10(6*H*)-dione (9)

A stirred suspension of 6-acetoxymethyl-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (8) (155.4 g, 0.41 mol), $K_2CO_3$ (68.1 g; 0.49 mol), and anhydrous methanol (4 L) was heated to 30°C. Oxygen was continuously passed through the reaction mixture for 3 h (then suspension became a solution as the reaction proceeded). TLC analysis (acetone/MeOH, 4:1) indicated completeness of reaction. The reaction mixture was acidified to pH 2 by the slow addition of 1M $H_2SO_4$ then concentrated in vacuo to a solid residue. This material was partitioned between $H_2O$ (6 L) and $CH_2Cl_2$ (2 L). The aqueous layer was removed and extracted with $CH_2Cl_2$ (3 x 3 L). The combined organic layers were dried over $Na_2SO_4$ then chromatographed over silica (6 kg) eluted with $CH_2Cl_2$/acetone (4:1) to give 39.9 g of high purity tricyclic ketal 9. Additional product (37.3 g) was obtained from another reaction. The material was suitable for further transformation.

(S)-2-Hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(S)-(-)-α-methylbenzyl]butyramide, monohydrate (10)

A solution of racemic (*R,S*)-4-ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3,10(6*H*)-dione (9) (77.2 g, 0.251 mol) and (S)-(-)-α-methylbenzylamine (381 g, 3.14 mol), maintained under an argon atmosphere, was heated at 75°C for 20 h then the excess amine was removed by vacuum distillation (333.2 g was recovered). The oily residue (132.5 g) was suspended in toluene (1.5 L), and the mixture was stirred for 2 h. The resulting solid was collected on a filter, washed with toluene, hexane, then dried; yield, 41.5 g (41.3%). The filtrate contains the more soluble (*R*)(*S*) diastereomer 10a. The material was suitable for further transformation.

(*S*)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano-[3,4-*f*]indolizine-3,10(6*H*)-dione (11)

A solution of (*S*)-2-hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(*S*)-(-)-α-methylbenzyl]butyramide (10) (41.2 g, 0.096 mol) and AcOH (1 L) was stirred at 75°C for 2 h then the solvent was removed by evaporation. The residue was dissolved in EtOAc-$CH_3OH$ (10:1) then chromatographed on a column of silica gel (500 g) using EtOAc-$CH_3OH$ (10:1) as the eluent. Fractions containing product were combined, concentrated in vacuo to give 28 g of crude 11. The material was recrystallized from EtOAc (870 mL) to give 24.6 g of pure 11. Additional product (11.0 g) was obtained previously. The material was suitable for further transformation.

(*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano-[3,4-*f*]indolizine-3,6,10-trione (12)

A mixture of (11) (16.9 g, 55 mmol), $H_2SO_4$ (16 mL), $H_2O$ (248 mL), and dioxane (390 mL) was heated under reflux for 80 min then cooled to RT. The mixture was extracted with $CH_2Cl_2$ (3 x 500 mL). The extracts were combined, then washed with $H_2O$ (2 x 200 mL). The water washes were combined, neutralized to pH 6 with solid $Na_2CO_3$ then extracted with $CH_2Cl_2$ (2 x 200 mL). The $CH_2Cl_2$ extracts and washes were combined, washed with $H_2O$ (200 mL), dried ($MgSO_4$), then concentrated in vacuo to give 10.1 g of amber colored residue. Additional crude product (15.1 g) was obtained from another reaction. The two lots were dissolved in $CH_3OH$ (~500 mL), charcoaled (2 g), then concentrated in vacuo to a yellow solid. The solid was dissolved in $CH_2Cl_2$ (200 mL) and hexane (200 mL) was added. The solid that separated was collected then stored. The filtrate was concentrated and the process was repeated. The solids were combined then dried (40°C); yield, 20.5 g (67.2%); mp 222-223°C; literature[18] mp 174-176°C; $[\alpha]_D^{20} = +103.8°$

(C=0.814; $CHCl_3$/MeOH 4:1); literature[18] $[\alpha]_D^{20}$= +105.2° (C=0.49; $CHCl_3$/MeOH 4:1). A portion (20.3 g) was transmitted to WRAIR on September 5, 1996 (Lot No. NJ24-38-1).

Anal.

| | | C | H | N |
|---|---|---|---|---|
| Calc'd for | $C_{13}H_{13}NO_5$ | 59.31 | 4.98 | 5.32 |
| | Found | 59.26 | 4.97 | 5.26 |

Spectral Data

Infrared (Nujol)

Major bands: 3400(b), 2900, 2840, 1730, 1645, 1500, 1445, 1405, 1390, 1370, 1330, 1300, 1250, 1220, 1185, 1140, 1080, 1045, 980, 935, 890, 810 $cm^{-1}$.

Ultraviolet (EtOH)

$\lambda_{max}$ 222 nm (sh, log $\varepsilon$ 4.05); 338 nm (3.90).

Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 7.22 (s, 1, pyridone H); 5.65 (d, 1, J= 17.1 Hz, H at C-1); 5.23 (d, 1, J= 17.1 Hz, H at C-1); 4.34 (t, 2, J=6.1 Hz, $COCH_2$); 3.84 (s, 1, OH); 2.96 (t, 2, J=5.2 Hz, $NCH_2$), 1.81 (d of q, 2, J= 7.1/2.2 Hz, $CH_3\underline{CH_2}$); 0.97 (t, 3, J=7.4 Hz, $CH_3$).

Source of Material

| | | |
|---|---|---|
| 1. | Sodium ethoxide in EtOH | Aldrich Chemical Co., Inc. |
| 2. | Ethanol | US Industrial Chem. Corp. |
| 3. | Diethyl oxalate | Aldrich Chemical Co., Inc. |
| 4. | Acetone | J.T. Baker Chemical Co. |
| 5. | Ether | Fisher Scientific |
| 6. | $H_2SO_4$ | J.T. Baker Chemical Co. |
| 7. | $CH_2Cl_2$ | J.T. Baker Chemical Co. |
| 8. | $NaHCO_3$ | Aldrich Chemical Co., Inc. |
| 9. | Ethyl acetopyruvate | Starks Associates, Inc. |
| 10. | Triethyl orthoformate | Aldrich Chemical Co., Inc. |
| 11. | $NH_4Cl$ | Aldrich Chemical Co., Inc. |
| 12. | $K_2CO_3$ | Aldrich Chemical Co., Inc. |
| 13. | Ethyl 2-ethoxy-4-oxo-pent-2-enoate | Starks Associates, Inc. |
| 14. | Cyanoacetamide | Aldrich Chemical Co., Inc. |
| 15. | DMF | Aldrich Chemical Co., Inc. |
| 16. | Methyl acrylate | Aldrich Chemical Co., Inc. |
| 17. | HCl | General Chemical Co. |
| 18. | AcOH | General Chemical Co. |
| 19. | $MgSO_4$ | J.T. Baker Chemical Co. |
| 20. | Hexane | J.T. Baker Chemical Co. |
| 21. | 6-Cyano-1,5-dioxo-7-methyl-1,2,3,5-tetrahydro-indolizine | Starks Associates, Inc. |
| 22. | Ethylene glycol | Aldrich Chemical Co., Inc. |
| 23. | Chlorotrimethylsilane | Aldrich Chemical Co., Inc. |
| 24. | NaOH | J.T. Baker Chemical Co. |

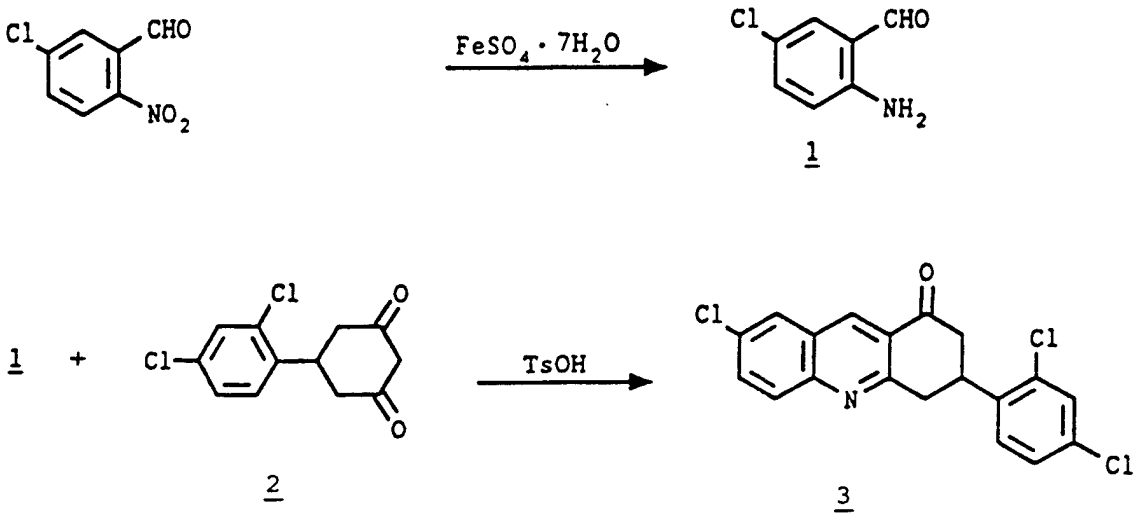
| | | |
|---|---|---|
| 25. | MeOH | J.T. Baker Chemical Co. |
| 26. | 6-Cyano-1,1-(ethylene-dioxy)-7-methyl-5-oxo-1,2,3,5-tetrahydro-indolizine | Starks Associates, Inc. |
| 27. | NaH | Lancaster Synthesis |
| 28. | Toluene | Aldrich Chemical Co., Inc. |
| 29. | Diethyl carbonate | Aldrich Chemical Co., Inc. |
| 30. | $Na_2SO_4$ | Aldrich Chemical Co., Inc. |
| 31. | Silica gel | EM Laboratories |
| 32. | 6-Cyano-7-[(ethoxycarbonyl)methyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine | Starks Associates, Inc. |
| 33. | 1,2-Dimethoxyethane | Aldrich Chemical Co., Inc. |
| 34. | Potassium *t*-butoxide | Aldrich Chemical Co., Inc. |
| 35. | Ethyl iodide | Aldrich Chemical Co., Inc. |
| 36. | EtOAc | J.T. Baker Chemical Co. |
| 37. | 6-Cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine | Starks Associates, Inc. |
| 38. | Raney Ni | Aldrich Chemical Co., Inc. |
| 39. | Acetic anhydride | Aldrich Chemical Co., Inc. |
| 40. | 6-Acetamidomethyl)-1,1-(ethylene-dioxy)-7-[1'-(ethoxycarbonyl)propyl]-5-oxo-1,2,3,5-tetrahydro-indolizine | Starks Associates, Inc. |
| 41. | Sodium nitrite | Aldrich Chemical Co., Inc. |
| 42. | $CCl_4$ | Aldrich Chemical Co., Inc. |
| 43. | 6-Acetoxymethyl)-7-[1'-(ethoxy-carbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydro-indolizine | Starks Associates, Inc. |
| 44. | $O_2$ | Linde |

| | | |
|---|---|---|
| 45. | (*RS*)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]-indolizine-3,10-(6*H*)-dione | Starks Associates, Inc. |
| 46. | (*S*)-(-)-α-Methylbenzylamine | Aldrich Chemical Co., Inc. |
| 47. | (*S*)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]-indolizine-3,10-(6*H*)-dione | Starks Associates, Inc. |
| 48. | Dioxane | J.T. Baker Chemical Co. |

11. 1(2*H*)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)- (3)

The target compound (3) was prepared by the following sequence of reactions.

Reaction Sequence:




Experimental

5-Chloro-2-aminobenzaldehyde (1)

To a boiling solution of ferrous sulfate, heptahydrate (53.0 g, 0.19 mol) in $H_2O$ (250 mL) was added a solution of 5-chloro-2-nitrobenzaldehyde (5.0 g, 26.9 mmol) in 50% aqueous ethanol (250 mL). The solution was boiled for one minute and then concentrated ammonium hydroxide (70 mL) was added in 10 mL portions. After the addition the suspension was boiled for 10 min, filtered hot, and the solid was washed with boiling $H_2O$ (2 x 100 mL). The filtrate and washings were combined, cooled, and the solid that separated was collected. This was dissolved in $CH_2Cl_2$ (100 mL), washed with $H_2O$ (50 mL),

dried over $Na_2SO_4$ and after removal of solvent in vacuo 2.2 g of 1 was obtained, which was suitable for the next step.

5-(2,4-Dichlorophenyl)cyclohexane-1,3-dione (2)

Please refer to Starks Associates, Inc. Annual Summary Report dated June 1987 (July 1, 1986 to June 30, 1987) Contract DAMD17-83-C-3206, pg. 28.

1(2*H*)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)- (3)

To a mixture of 5-chloro-2-aminobenzaldehyde (1) (2.2 g, 14.1 mmol) and 5-(2,4-dichlorophenyl)cyclohexane-1,3-dione (2) (3.65 g, 14.2 mmol) in toluene (1 L) was added *p*-toluenesulfonic acid monohydrate (200 mg) and the mixture was heated at slow reflux using Dean Stark apparatus to remove water. After 2 h of reflux the reaction was cooled and concentrated in vacuo. The residue was triturated with hot $CH_3OH$ (100 mL). The solid was collected then crystallized from glacial acetic acid (150 mL); yield 1.7 g (32.0%) mp 222-223°C. A portion (200 mg) was transmitted to WRAIR on October 18, 1996 (NJ24-38-1).

Anal.

| | | C | H | N |
|---|---|---|---|---|
| Calc'd for | $C_{19}H_{12}Cl_3NO$ | 60.59 | 3.21 | 3.72 |
| | Found | 60.33 | 3.35 | 3.63 |

Spectral Data

Infrared (KBr)

Major bands: 3430, 3060, 1690, 1610, 1590, 1555, 1475, 1450, 1430, 1395, 1370, 1300, 1270, 1230, 1205, 1190, 1130, 1100, 1070, 1050, 1015, 930 $cm^{-1}$.

Ultraviolet ($CHCl_3$)

$\lambda_{max}$ 260 nm (log $\varepsilon$ 4.75); 325 nm (3.93).

Nuclear Magnetic Resonance (TFA-d)

$\delta$ 9.71 (s, 1, H-9); 8.46 (s, 1, H-8); 8.31 (m, 2, H-5 and H-6); 7.53 (d, 1, C-3'), 7.38 (m, 1, C-5'); 7.34 (d, 1, C-6'); 4,37 (m, 1, C-3); 3.97 (m, 2, C-4); 3.40 (m, 2, C-2).

Mass Spectrometry (Electron impact)

m/e [Rel. int., ID]; 379 [11, $(m+4)^+$]; 378 [10, $(m + 3)^+$]; 377 [32, $(m + 2)^+$]; 375 [32, $m^+$].

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; visulization - UV.

| Eluent | Rf Value | Comment |
|---|---|---|
| Hexane-acetone (4:1) | 0.36 | Single spot |
| Hexane-EtOAc (2:1) | 0.74 | Single spot |

Source of Material

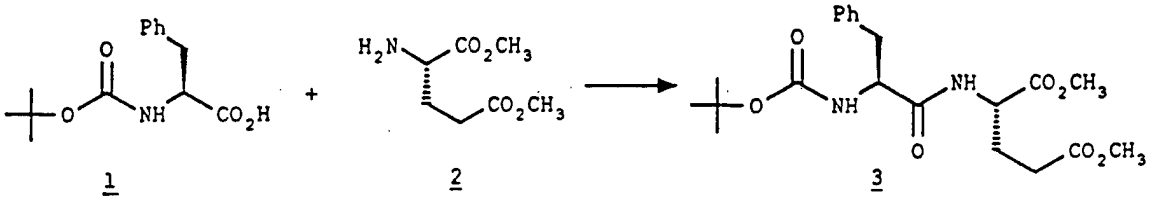
| | | |
|---|---|---|
| 1. | 5-Chloro-2-nitrobenzaldehyde | Aldrich Chemical Co., Inc. |
| 2. | $FeSO_4 \cdot 7\ H_2O$ | Sigma |
| 3. | $CH_2Cl_2$ | J.T. Baker Chemical Co. |
| 4. | $Na_2SO_4$ | J.T. Baker Chemical Co. |
| 5. | 5-(2,4-Dichlorophenyl)-cyclohexane-1,3-dione | Starks Associates, Inc. |
| 6. | Toluene | J.T. Baker Chemical Co. |
| 7. | *p*-Toluenesulfonic acid | Aldrich Chemical Co., Inc. |
| 8. | Methanol | J.T. Baker Chemical Co. |
| 9. | Acetic acid | J.T. Baker Chemical Co. |

B. Chemical Defense Related Compounds and Intermediates

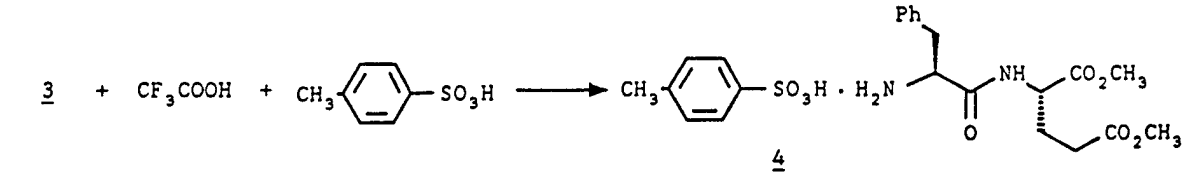
12. *L*-Glutamic acid, N-[N-[ethoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt (9)

The target compound 9 was prepared by the following reaction sequence.

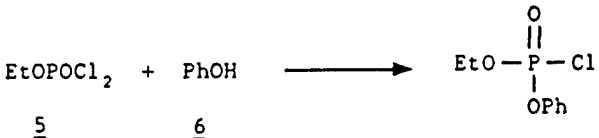
Reaction Sequence:

a. 

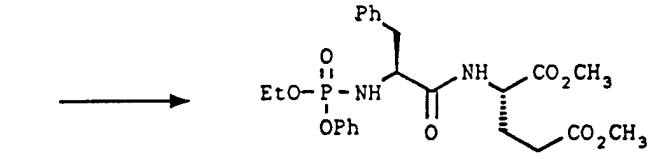
1 2 3

b. 

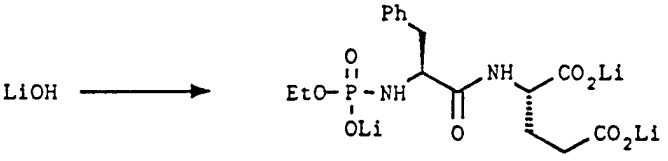
4

c. 

5 6 7

d. 

8

e. 

9

## Experimental[5]

*L*-Glutamic acid, N-[N-[*tert*-butoxycarbonyl]-*L*-phenylalanyl]-, dimethyl ester (3)

To a stirring suspension of N-α-*t*-butoxycarbonyl-*L*-phenylalanine (1) (5.0 g, 18.85 mmol), *L*-glutamic acid dimethyl ester hydrochloride (2) (4.8 g, 22.62 mmol), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (7.15 g, 18.85 mmol), and N-hydroxybenzotriazole hydrate ($HOBt \cdot H_2O$) (2.55 g, 18.85 mmol) in dichloromethane (52 mL), at 7-10°C under argon, was added dropwise N,N-diisopropylethylamine (14.8 mL, 84.8 mmol), while maintaining an internal temperature of 10-12°C. After addition, the mixture was stirred at ambient temperature for 22 h. The mixture was concentrated to an oil, then dissolved in ethyl acetate (100 mL) and washed with 5% HCl (aq) (2 x 25 mL), $H_2O$ (2 x 50 mL), sat. $NaHCO_3$ (aq) (2 x 50 mL), and brine (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to give a light yellow oil (11 g), which was purified by flash column chromatography (300 g of flash $SiO_2$) eluted with 2:3 EtOAc:hexanes to give 6.1 g (77%) of pure 3.

Spectral Data:

Nuclear Magnetic Resonance ($CDCl_3$)

δ 7.31-7.18 (m, 5H, Ar); 6.52-6.50 (m, 1H); 5.11-4.79 (m, 1H); 4.56-4.46 (m, 1H); 4.40-4.30 (m, 1H); 3.70 (s, 3H); 3.66 (s, 3H); 3.08-3.04 (m, 2H); 2.20-2.15 (m, 3H); 1.95-1.91 (m, 1H); 1.40 (s, 9H).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - anisaldehyde spray.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc-hexanes (2:3) | 0.29 | Homogeneous |

*L*-Glutamic acid, N-[*L*-phenylalanyl]-, dimethyl ester tosylate (4)

To an ice-chilled flask containing *L*-glutamic acid, N-[N-[*tert*-butoxycarbonyl]-*L*-phenylalanyl]-, dimethyl ester (4.9 g, 11.6 mmol) was added ice-cold trifluoroacetic acid (27 mL) with stirring under argon. The mixture was stirred at 0°C for 15 min, then *p*-toluenesulfonic acid monohydrate (2.2 g, 11.6 mmol) was added and the mixture was stirred at ambient temperature for 1 h. Concentrated to give an oil which was then coevaporated with $CH_2Cl_2$ (150 mL). The resulting oil was triturated with *tert*-butyl methyl ether (100 mL) and stirred for 1 h. The suspension was suction filtered and the filter cake was washed with $Et_2O$ (150 mL) and dried in vacuo to give pure 4 (5.6 g, 98%) as a white solid. An additional reaction was performed to give a total of 6.8 g of pure 4.

Spectral Data:

Nuclear Magnetic Resonance

$\delta$ 7.95-7.72 (m, 4H, Ar); 7.14 (s, 5H, Ar); 4.70 (m, 1H); 4.38 (m, 1H); 3.63 (s, 3H); 3.41 (s, 3H); 3.30-3.19 (m, 2H); 2.34 (s, 3H); 2.33-2.18 (m, 2H); 2.05-1.80 (m, 2H).

*L*-Glutamic acid, N-[N-[ethoxyphenoxyphosphinyl]-*L*-phenylalanyl]-, dimethyl ester (8)

To a solution of ethyl dichlorophosphate (5) (1.44 mL, 12.13 mmol) in $CH_2Cl_2$ (240 mL) at 0°C under argon was added a solution of anhydrous phenol (6) (1.2 g, 12.74 mmol) and triethylamine (1.8 mL, 12.9 mmol) in $CH_2Cl_2$ (120 mL) over 0.5 h. The mixture was stirred at 0°C/0.5 h and then at ambient temperature for 2 h, to give a solution of 7. To this solution was added a suspension of *L*-glutamic acid, N-[*L*-phenylalanyl]-, dimethyl ester tosylate (6.3 g, 12.74 mmol), triethylamine (1.9 mL, 13.63 mmol), and 4-dimethylaminopyridine (0.147 g, 1.20 mmol) in anhydrous THF (120 mL). The suspension was stirred at ambient temperature for 20 h, then concentrated to a residue which was dissolved in EtOAc (500 mL) and washed with 0.1N HCl (2 x 250 mL), sat. $NaHCO_3$ (2 x 250 mL), 50% NaCl (2 x 250 mL), and brine (250 mL). The organic layer was dried ($Na_2SO_4$), filtered, and concentrated to give a crude product (4.2 g) which was purified by flash chromatography (250 g flash $SiO_2$, 3:1 EtOAc:hexanes) to give pure 8, as a mixture of diastereomers (1.6 g, 26%).

Spectral Data:

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

Diastereomeric mixture:
$\delta$ 7.35-7.10 (m, 10H); 6.92-6.90 (2d, 1H); 4.56-4.50 (m, 1H); 4.13-3.90 (2m, 3H); 3.69-3.64 (4s, 6H); 3.38-3.15 (2m, 2H); 2.98-2.82 (2m, 1H); 2.26-2.11 (m, 3H); 1.89-1.87 (m, 1H); 1.29-1.26 (t, 3H).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

Diastereomeric mixture:

$\delta$ 2.86 (s, 1P); 2.75 (s, 1P).

Mass Spectrum

Method of Ionization = Electrospray (positive)

Calc'd for $C_{24}H_{31}N_2O_8P$ = 506.2

Found: 507.4 (m + H)

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - anisaldehyde spray.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc-hexanes (3:1) | 0.28 | Homogeneous |

L-Glutamic acid, N-[N-[ethoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt (9)

To a solution of *L*-glutamic acid, N-[N-[ethoxyphenoxyphosphinyl]-*L*-phenylalanyl]-, dimethyl ester (1.6 g, 3.16 mmol) in THF (160 mL) and $H_2O$ (80 mL) was added 1.5M LiOH (9.8 mL, 14.7 mmol), and the solution was refluxed for 8 h. After cooling to room temperature, 0.985N HCl (5.3 mL) was added and the solvents were evaporated at reduced pressure. Water (3 x 160 mL) was added and removed at reduced pressure (for azeotropic removal of phenol). The residue was coevaporated with anhydrous $CH_3CN$ (3 x 80 mL) (for azeotropic removal of

$H_2O$), then pumped dry in vacuo to give 9, as a white solid (1.5 g, 88%, based on a molecular formula of $C_{16}H_{20}Li_3N_2O_8P$ · 1.7 LiCl·2.75 $H_2O$). A portion (1.0 g) was transmitted to WRAIR on January 11, 1996 (Lot No. NJ20-27-1).

Anal.

| | C | H |
|---|---|---|
| Calc'd for $C_{16}H_{20}Li_3N_2O_8P$ ·1.7 LiCl·2.75 $H_2O$ | 35.47 | 4.74 |
| Found | 35.49 | 4.70 |
| | 35.40 | 4.72 |

Spectral Data

$^1$H Nuclear Magnetic Resonance ($D_2O$)

δ 7.25-7.13 (m, 5H, Ar); 4.00-3.98 (dd, 1H, C<u>H</u>); 3.80-3.78 (dd, 1H, C<u>H</u>); 3.58-3.47 (m, 2H, $CH_3$<u>$CH_2$</u>); 3.00-2.88 (m, 2H, Ph<u>$CH_2$</u>); 1.88-1.81 (m, 3H, <u>CH</u>-<u>$CH_2$</u>-CO); 1.74-1.72 (m, 1H, <u>CH</u>-C-CO); 1.00-0.97 (t, 3H, <u>$CH_3$</u>$CH_2$).

$^{31}$P Nuclear Magnetic Resonance ($D_2O$)

δ 6.56 (s, 1P).

Mass Spectrum

Method of Ionization = Electrospray (negative)
Calc'd for $C_{16}H_{23}N_2O_8P$ = 402.12 (free acid)
Found: 401.4 (m-H), 407.2 (m-2H+Li),
413.4 (m-3H+2Li).

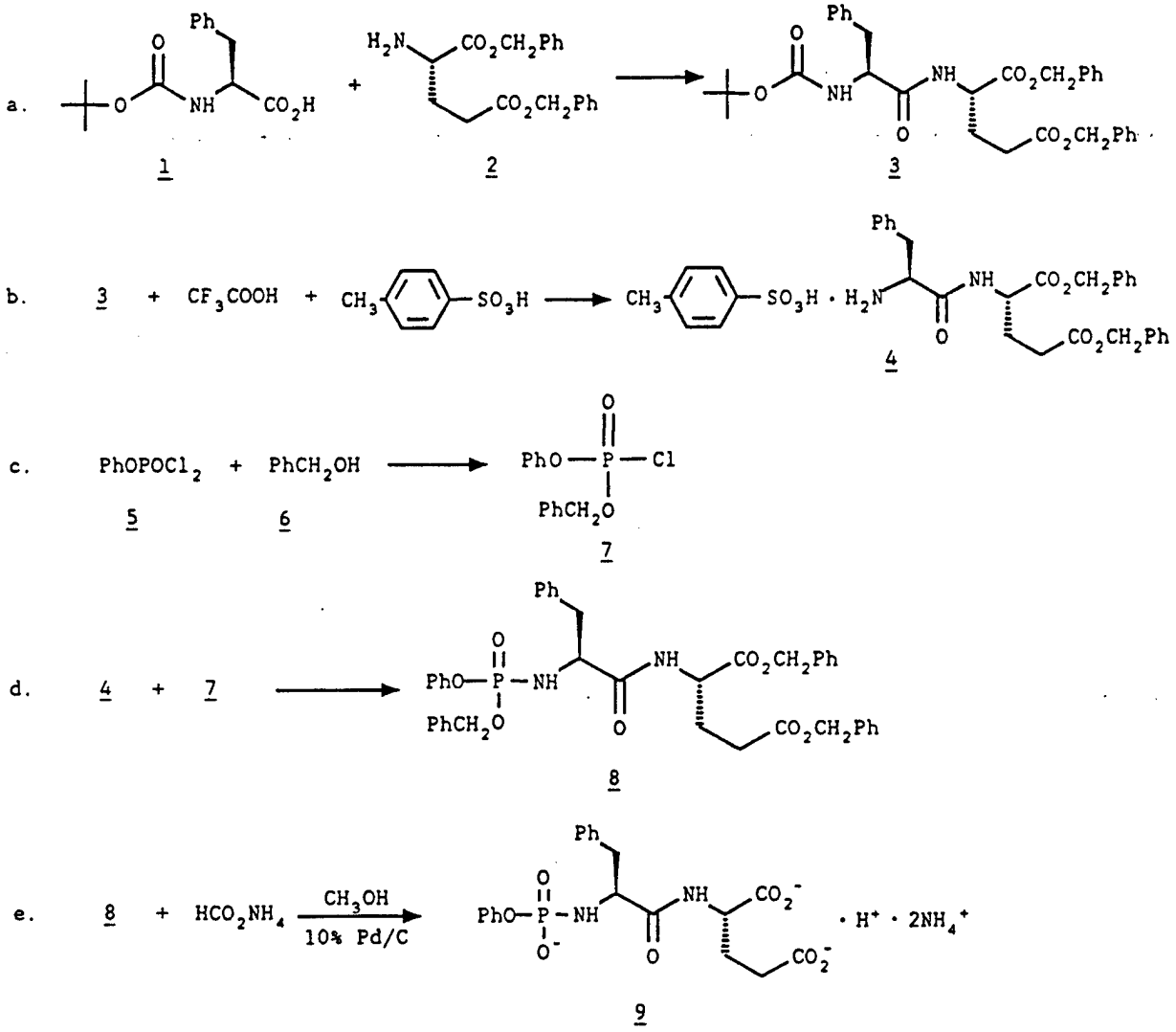
Source of Materials

| | | |
|---|---|---|
| 1. | N-α-*t*-butoxycarbonyl-*L*-phenylalanine | Nova Biochem |
| 2. | *L*-Glutamic acid dimethyl ester hydrochloride | Sigma Chemical Co. |
| 3. | HBTU | Nova Biochem |
| 4. | $HOBt \cdot H_2O$ | Nova Biochem |
| 5. | N,N-Diisopropylethylamine | Aldrich Chemical Co., Inc. |
| 6. | Dichloromethane | J.T. Baker Chemical Co. |
| 7. | EtOAc | EM Science |
| 8. | Hexanes | EM Science |
| 9. | Hydrochloric acid | J.T. Baker Chemical Co. |
| 10. | Sodium bicarbonate | J.T. Baker Chemical Co. |
| 11. | Magnesium sulfate | J.T. Baker Chemical Co. |
| 12. | Flash silica gel | E.M. Science |
| 13. | Trifluoroacetic acid | Aldrich Chemical Co., Inc. |
| 14. | *p*-Toluenesulfonic acid monohydrate | Kodak |
| 15. | *tert*-Butyl methyl ether | Aldrich Chemical Co., Inc. |
| 16. | Diethyl ether | Fisher Scientific |
| 17. | Ethyl dichlorophosphate | Aldrich Chemical Co., Inc. |
| 18. | Phenol | Aldrich Chemical Co., Inc. |
| 19. | Triethylamine | Aldrich Chemical Co., Inc. |
| 20. | 4-Dimethylaminopyridine | Reilly Industries |
| 21. | THF | Aldrich Chemical Co., Inc. |
| 22. | NaCl | Cargill |
| 23. | Sodium sulfate | Aldrich Chemical Co., Inc. |
| 24. | LiOH | Aldrich Chemical Co., Inc. |
| 25. | Hydrochloric acid, 0.985N | Aldrich Chemical Co., Inc. |
| 26. | $CH_3CN$ | Aldrich Chemical Co., Inc. |

13. *L*-Glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, diammonium salt (9)

The target compound 9 was prepared by the following reaction sequence.

Reaction Sequence:



## Experimental[5,6]

*L*-Glutamic acid, N-[N-[*tert*-butoxycarbonyl]-*L*-phenylalanyl]-, dibenzyl ester (3)

To a stirring suspension of N-α-*t*-butoxycarbonyl-*L*-phenylalanine (1) (5 g, 11.3 mmol), *L*-glutamic acid, α,γ-dibenzyl ester tosylate (2) (11.3 g, 22.62 mmol), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (7.15 g, 18.85 mmol), and N-hydroxybenzotriazole hydrate ($HOBt \cdot H_2O$) (2.55 g, 18.85 mmol) in dichloromethane (52 mL), at 7-10°C under argon, was added dropwise N,N-diisopropylethylamine (14.8 mL, 84.8 mmol), while maintaining an internal temperature of 10-12°C. After addition, the mixture was stirred at ambient temperature for 22 h. The mixture was concentrated to an oil, then dissolved in ethyl acetate (250 mL) and washed with 5% HCl (aq) (2 x 100 mL), $H_2O$ (2 x 100 mL), sat. $NaHCO_3$ (aq) (2 x 100 mL), and brine (200 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to give a solid which was triturated and suction filtered with $t$-$BuOCH_3$ (120 mL) for 1 h. Dried in vacuo to give pure 3 (9.7 g, 90%).

Spectral Data:

Nuclear Magnetic Resonance ($CDCl_3$)

δ 7.35-7.15 (m, 15H, Ar); 6.54-6.52 (m, 1H); 5.16-5.07 (m, 3H); 4.90 (m, 1H); 4.61 (m, 1H); 4.33 (m, 1H); 3.05-3.02 (m, 2H); 2.34-2.18 (m, 3H); 1.96-1.94 (m, 1H); 1.38 (s, 9H).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - anisaldehyde spray.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc-hexanes (1:2) | 0.42 | Homogeneous |

*L*-Glutamic acid, N-[*L*-phenylalanyl]-, dibenzyl ester tosylate (4)

To an ice-chilled flask containing *L*-glutamic acid, N-[N-[*tert*-butoxycarbonyl]-*L*-phenylalanyl]-, dibenzyl ester (8.7 g, 15.14 mmol) was added ice-cold trifluoroacetic acid (40 mL) with stirring under argon. The mixture was stirred at 0°C for 30 min, then *p*-toluenesulfonic acid monohydrate (2.88 g, 15.14 mmol) was added and the mixture was stirred at ambient temperature for 1 h. Concentrated to give an oil which was then coevaporated with $CH_2Cl_2$ (150 mL). The residue was triturated with *tert*-butyl methyl ether (200 mL) and stirred for 1 h. The suspension was suction filtered and the filter cake was washed with $Et_2O$ (50 mL) and then dried in vacuo to give pure 4 (9.6 g, 98%) as a white solid.

Spectral Data:

Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 7.92-7.03 (m, 19H, Ar); 5.04 (s, 2H, $PhCH_2$); 4.85 (s, 2H, $PhCH_2$); 4.66 (m, 1H, CH); 4.41 (m, 1H, CH); 3.40-3.35 (br m, 1H); 3.25-3.10 (m, 2H, $PhCH_2$); 2.27-2.17 (m, 2H); 2.25 (s, 3H); 2.00-1.87 (m, 2H).

*L*-Glutamic acid, N-[N-[benzyloxyphenoxyphosphinyl]-*L*-phenylalanyl]-, dibenzyl ester (8)

To a solution of phenyl dichlorophosphate (5) (2.2 mL, 14.7 mmol) in $CH_2Cl_2$ (300 mL) at 0°C under argon was added a solution of benzyl alcohol (6) (1.7 mL, 16.4 mmol) and triethylamine (2.9 mL, 20.8 mmol) in $CH_2Cl_2$ (150 mL) over 0.5 h. The mixture was stirred at 0°C/0.5 h and then at ambient temperature for 2 h, to give a solution of 7. To this solution was added a suspension of *L*-glutamic acid, N-[*L*-phenylalanyl]-, dibenzyl ester tosylate (9.5 g, 14.7 mmol), triethylamine (7.2 mL, 51.7 mmol), and 4-dimethylaminopyridine (287 mg, 2.35 mmol) in anhydrous THF (150 mL). The suspension was stirred at ambient temperature for 22 h, then concentrated to a residue which was dissolved in EtOAc (1 L) and washed with 0.1N HCl (2 x 500 mL), sat. $NaHCO_3$ (2 x 500 mL), 50% NaCl (2 x 500 mL), and brine (500 mL). The organic layer was dried ($Na_2SO_4$), filtered, and concentrated to give a crude product (10.6 g) which was purified by flash chromatography (300 g, flash $SiO_2$, 1:1 EtOAc:hexanes) to give pure 8, as a mixture of diastereomers (2.3 g, 23%).

Spectral Data:

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

Diastereomeric mixture:
$\delta$ 7.37-6.79 (m, 25H); 5.08-5.00 (m, 7H); 4.90 and 4.70 (2m, 1H); 4.56-4.53 (m, 1H); 3.33-3.29 (m, 1H); 3.26-3.13 (2m, 1H); 2.87-2.82 (m, 1H); 2.23-2.10 (m, 3H); 1.88-1.84 (m, 1H).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

Diastereomeric mixture:

$\delta$ 2.99 (s, 1P); 2.97 (s, 1P).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - $I_2$.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc:hexanes (1:1) | 0.32 | Homogeneous |

*L*-Glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, diammonium salt (9)

A mixture of *L*-glutamic acid, N-[N-[benzyloxyphenoxyphosphinyl]-*L*-phenylalanyl]-, dibenzyl ester (1.8 g, 2.50 mmol), ammonium formate (2.1 g, 33.3 mmol) and 10% palladium on carbon (1.8 g) in methanol (45 mL) was stirred at ambient temperature, under argon, for 24 h. The mixture was filtered through a pad of celite and concentrated to give a residue, which was dissolved in $H_2O$ (25 mL) and again filtered through a pad of celite. The aqueous filtrate was concentrated in vacuo and the white solid residue was coevaporated with $H_2O$ (12 x 35 mL) to azeotropically remove ammonium formate. The residue was then coevaporated with $CH_3CN$ (3 x 50 mL), to azeotropically remove $H_2O$, to give 9 (0.95 g, 76% based on a molecular formula of $C_{20}H_{29}N_4O_8P \cdot 0.8H_2O$). A portion (720 mg) was transmitted to WRAIR on January 30, 1996 (Lot No. NJ20-45-1).

Anal.

| | C | H | N |
|---|---|---|---|
| Calc'd for $C_{20}H_{29}N_4O_8P \cdot 0.8\ H_2O$ | 48.15 | 6.18 | 11.23 |
| Found | 48.15 | 5.77 | 10.61 |
| | 48.05 | 5.82 | 10.58 |

Spectral Data

FT-Infrared (KBr)

Major bands: 3296, 3227, 3062, 3028, 2921, 2857, 1932, 1707, 1645, 1592, 1542, 1492, 1453, 1408, 1320, 1255, 1202, 1069, 889, 764, 545 $cm^{-1}$.

$^1$H Nuclear Magnetic Resonance ($D_2O$)

$\delta$ 7.23-6.97 (m, 10H, Ar); 3.94-3.85 (2m, 2H, 2C<u>H</u>); 2.97-2.75 (m, 2H, Ph<u>C</u>$H_2$); 1.97-1.94 (m, 2H, C-<u>C</u>$H_2$-CO); 1.84-1.64 (2m, 2H, -<u>C</u>$H_2$-C-CO).

A trace of ammonium formate is also seen at $\delta$ 8.28.

$^{31}$P Nuclear Magnetic Resonance ($D_2O$)

$\delta$ 2.67 (s, 1P).

Mass Spectrum

Method of Ionization = Electrospray (negative)
Calc'd for $C_{20}H_{23}N_2O_8P$ = 450.1 (free acid)
Found: 449.2 (m-H), 355.1 (m-2H-PhO).

Source of Materials

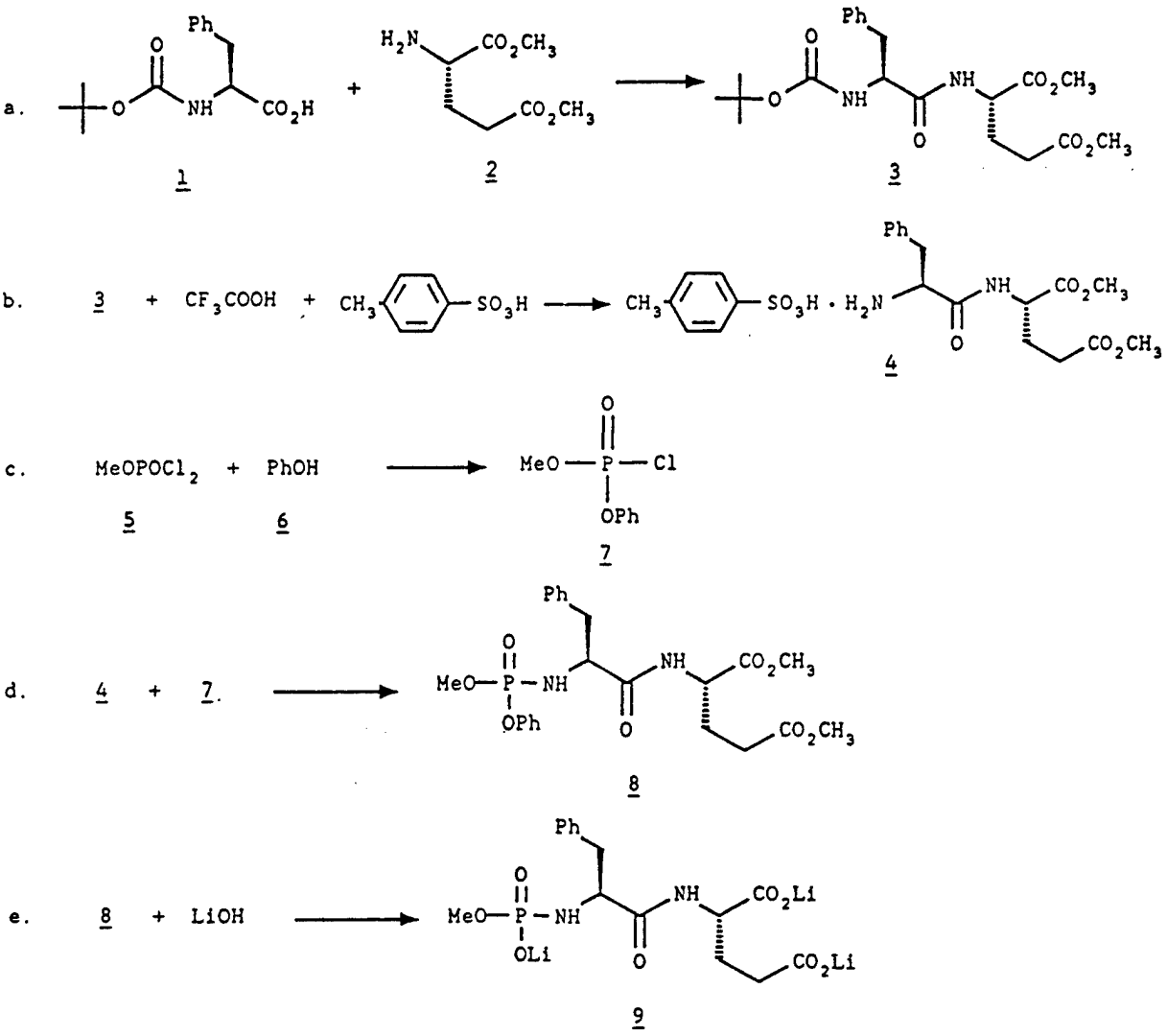
| | | |
|---|---|---|
| 1. | N-α-*t*-butoxycarbonyl-*L*-phenylalanine | Nova Biochem |
| 2. | *L*-Glutamic acid, α,γ-dibenzyl-ester tosylate | Bachem Bioscience Inc. |
| 3. | HBTU | Nova Biochem |
| 4. | HOBt·$H_2O$ | Nova Biochem |
| 5. | N,N-Diisopropylethylamine | Aldrich Chemical Co., Inc. |
| 6. | Dichloromethane | J.T. Baker Chemical Co., Aldrich Chemical Co., Inc. |
| 7. | EtOAc | EM Science |
| 8. | Hexanes | EM Science |
| 9. | Hydrochloric acid | J.T. Baker Chemical Co. |
| 10. | Sodium bicarbonate | J.T. Baker Chemical Co. |
| 11. | Magnesium sulfate | J.T. Baker Chemical Co. |
| 12. | Flash silica gel | E.M. Science |
| 13. | Trifluoroacetic acid | Aldrich Chemical Co., Inc. |
| 14. | *p*-Toluenesulfonic acid monohydrate | Kodak |
| 15. | *tert*-Butyl methyl ether | Aldrich Chemical Co., Inc. |
| 16. | Diethyl ether | Fisher Scientific |
| 17. | Phenyl dichlorophosphate | Aldrich Chemical Co., Inc. |
| 18. | Benzyl alcohol | Aldrich Chemical Co., Inc. |
| 19. | Triethylamine | Aldrich Chemical Co., Inc. |
| 20. | 4-Dimethylaminopyridine | Reilly Industries |
| 21. | THF | Aldrich Chemical Co., Inc. |
| 22. | NaCl | Cargill |
| 23. | Sodium sulfate | Aldrich Chemical Co., Inc. |
| 24. | Ammonium formate | Aldrich Chemical Co., Inc. |

| | | |
|---|---|---|
| 25. | $CH_3OH$ | J.T. Baker Chemical Co. |
| 26. | $CH_3CN$ | Aldrich Chemical Co., Inc. |
| 27. | 10% Pallacdium on carbon | Aldrich Chemical Co., Inc. |

## 14. L-Glutamic acid, N-[N-[methoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt (9)

The target compound 9 was prepared by the following reaction sequence.

Reaction Sequence:



## Experimental[5]

*L*-Glutamic acid, N-[N-[*tert*-butoxycarbonyl]-*L*-phenylalanyl]-, dimethyl ester (3)

To a stirring suspension of N-α-*t*-butoxycarbonyl-*L*-phenylalanine (1) (10.0 g, 37.7 mmol), *L*-glutamic acid dimethyl ester hydrochloride (2) (9.6 g, 45.3 mmol), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (14.3 g, 37.7 mmol), and N-hydroxybenzotriazole hydrate ($HOBt \cdot H_2O$) (5.1 g, 37.7 mmol) in dichloromethane (106 mL), at 7-10°C under argon, was added dropwise N,N-diisopropylethylamine (29.6 mL, 170 mmol), while maintaining an internal temperature of 10-12°C. After addition, the mixture was stirred at ambient temperature for 22 h. The mixture was concentrated to an oil, then dissolved in ethyl acetate (200 mL) and washed with 5% HCl (aq) (3 x 100 mL), $H_2O$ (2 x 100 mL), sat. $NaHCO_3$ (aq) (2 x 100 mL), and brine (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to give a solid (15.3 g), which was triturated with *t*-$BuOCH_3$ (30 mL) for 1 h, then diluted with pet ether (100 mL) and suction filtered and dried in vacuo to give pure 3 (14.4 g, 91%).

Spectral Data:

Nuclear Magnetic Resonance ($CDCl_3$)

δ 7.31-7.18 (m, 5H, Ar); 6.52-6.50 (m, 1H); 4.96-4.94 (m, 1H); 4.58-4.53 (m, 1H); 4.35-4.33 (m, 1H); 3.70 (s, 3H); 3.66 (s, 3H); 3.08-3.01 (m, 2H); 2.36-2.14 (m, 3H); 1.98-1.90 (m, 1H); 1.41 (s, 9H).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - anisaldehyde spray.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc-hexanes (1:1) | 0.51 | Homogeneous |

*L*-Glutamic acid, N-[*L*-phenylalanyl]-, dimethyl ester tosylate (4)

To an ice-chilled flask containing *L*-glutamic acid, N-[N-[*tert*-butoxycarbonyl]-*L*-phenylalanyl]-, dimethyl ester (14.4 g, 34.1 mmol) was added ice-cold trifluoroacetic acid (80 mL) with stirring under argon. The mixture was stirred at 0°C for 30 min, then *p*-toluenesulfonic acid monohydrate (6.49 g, 34.1 mmol) was added and the mixture was stirred at ambient temperature for 1 h. Concentrated to give an oil which was then coevaporated with $CH_2Cl_2$ (2x200 mL). The resulting oil was triturated with *tert*-butyl methyl ether (200 mL) and stirred for 1 h. The suspension was suction filtered and the filter cake was washed with $Et_2O$ (2x50 mL) and dried in vacuo to give pure 4 (16.5 g, 98%) as a white solid.

Spectral Data:

Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 7.93-7.72 (m, 4H, Ar); 7.14 (s, 5H, Ar); 4.70 (m, 1H); 4.38 (m, 1H); 3.62 (s, 3H); 3.42 (s, 3H); 3.30-3.14 (m, 2H); 2.34 (s, 3H); 2.32-2.18 (m, 2H); 2.01-1.85 (m, 2H).

*L*-Glutamic acid, N-[N-[methoxyphenoxyphosphinyl]-*L*-phenylalanyl]-, dimethyl ester (8)

To a solution of methyl dichlorophosphate (5) (1.0 mL, 10.0 mmol) in $CH_2Cl_2$ (200 mL) at 0°C under argon was added a solution of anhydrous phenol (6) (0.99 g, 10.5 mmol) and triethylamine (1.54 mL, 11.0 mmol) in $CH_2Cl_2$ (100 mL) over 45 min. The mixture was stirred at 0°C/2 h to give a solution of 7. To this solution at 0°C was added a suspension of *L*-glutamic acid, N-[*L*-phenylalanyl]-, dimethyl ester tosylate (5.19 g, 10.5 mmol), triethylamine (1.54 mL, 11.0 mmol), and 4-dimethylaminopyridine (0.122 g, 1.0 mmol) in anhydrous THF (100 mL). The suspension was warmed to ambient temperature and stirred for 20 h, then concentrated to a residue which was dissolved in EtOAc (1 L) and washed with 0.1N HCl (2 x 200 mL), sat. $NaHCO_3$ (2 x 200 mL), 50% NaCl (2 x 200 mL), and brine (200 mL). The organic layer was dried ($Na_2SO_4$), filtered, and concentrated to give a crude product (3.1 g) which was purified by column chromatography (90 g, $SiO_2$, EtOAc) to give pure 8, as a mixture of diastereomers (0.9 g, 18%). An additional reaction was performed to give a total of 1.8 g of pure 8.

Spectral Data:

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

Diastereomeric mixture:
$\delta$ 7.33-7.04 (m, 11H); 4.55-4.53 (m, 1H); 4.12-4.10 (m, 1H); 3.73-3.63 (m, 9H); 3.57-3.55 (d, 1H); 3.21-3.10 (m, 1H); 3.08-2.90 (m, 1H); 2.28-2.10 (m, 3H); 1.90-1.87 (m, 1H).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

Diastereomeric mixture:

$\delta$ 4.12 (s, 1P); 3.97 (s, 1P).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - $I_2$.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc | 0.46 | Homogeneous |

*L*-Glutamic acid, N-[N-[methoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt (9)

To a solution of *L*-glutamic acid, N-[N-[methoxyphenoxyphosphinyl]-*L*-phenylalanyl]-, dimethyl ester (1.62 g, 3.29 mmol) in THF (162 mL) and $H_2O$ (81 mL) was added 1.5M LiOH (10.2 mL, 15.3 mmol), and the solution was refluxed for 8 h. After cooling to room temperature, 0.985N HCl (5.513 mL) was added and the solvents were evaporated at reduced pressure. Water (3 x 160 mL) was added and removed at reduced pressure (for azeotropic removal of phenol). The residue was coevaporated with anhydrous $CH_3CN$ (3 x 100 mL) (for azeotropic removal of $H_2O$), then pumped dry in vacuo to give 9, as a white solid (1.6 g, 92%, based on a molecular formula of $C_{15}H_{18}Li_3N_2O_8P$ $0.55CH_3CN \cdot 1.5$ LiCl$\cdot 1.9$ $H_2O$). A portion (820 mg) was transmitted to WRAIR on February 7, 1996 (Lot No. NJ20-57-1).

Anal.

| | C | H | N |
|---|---|---|---|
| Calc'd for $C_{15}H_{18}Li_3N_2O_8P$ | 36.73 | 4.49 | 6.78 |
| $\cdot 0.55\ CH_3CN \cdot 1.5LiCl \cdot 1.9H_2O$ | | | |
| Found | 36.78 | 4.53 | 6.82 |
| | 36.68 | 4.54 | 6.78 |

Spectral Data

FT-Infrared (KBr)

Major bands: 3383, 2950, 2849, 2361, 1616, 1538, 1420, 1211, 1087, 1054, 961, 913, 841, 776, 702, 542 $cm^{-1}$.

$^1$H Nuclear Magnetic Resonance ($D_2O$)

$\delta$ 7.28-7.09 (m, 5H, Ar); 4.03-4.01 (dd, 1H, C<u>H</u>); 3.84-3.80 (dd, 1H, C<u>H</u>); 3.22-3.20 (m, 3H, <u>$CH_3$</u>O); 3.01-2.83 (m, 2H, Ph<u>$CH_2$</u>); 1.96 ($CH_3CN$); 1.93-1.85 (m, 3H, <u>CH</u>-<u>$CH_2$</u>CO); 1.77-1.73 (m, 1H, <u>CH</u>-C-CO).

$^{31}$P Nuclear Magnetic Resonance ($D_2O$)

$\delta$ 7.74 (s, 1P).

Mass Spectrum

Method of Ionization = Electrospray (negative)
Calc'd for $C_{15}H_{21}N_2O_8P$ = 388.1 (free acid)
Found: 387.0 (m-H), 393.2 (m-2H+Li), 399.2 (m-3H+2Li).

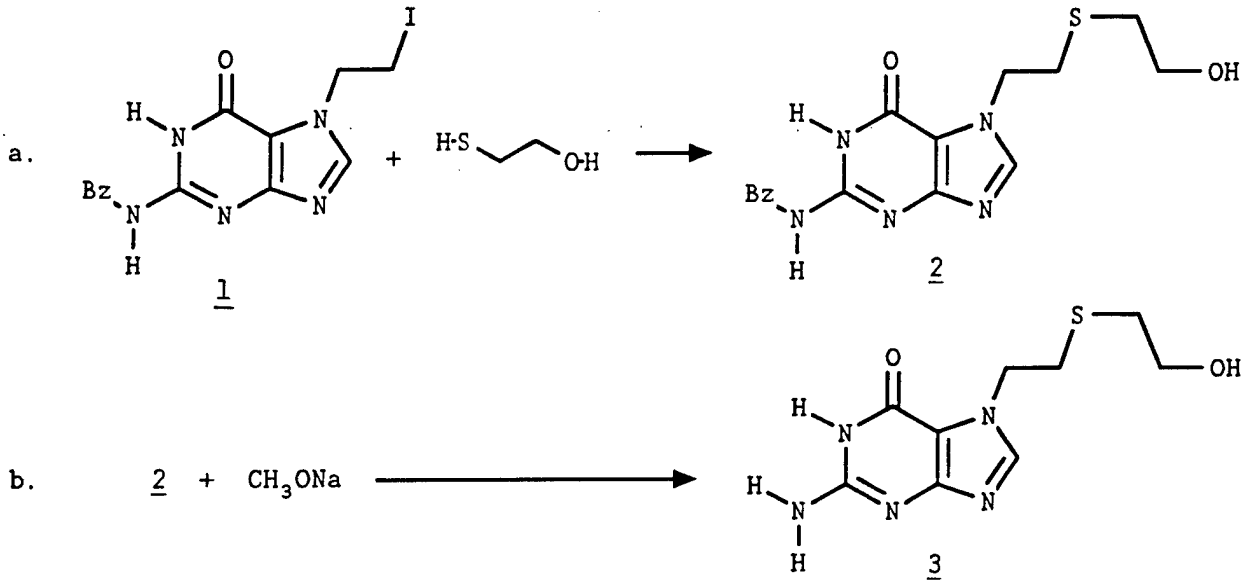
Source of Materials

| | | |
|---|---|---|
| 1. | N-α-*t*-butoxycarbonyl-*L*-phenylalanine | Nova Biochem |
| 2. | *L*-Glutamic acid dimethyl ester hydrochloride | Sigma Chemical Co. |
| 3. | HBTU | Nova Biochem |
| 4. | $HOBt \cdot H_2O$ | Nova Biochem |
| 5. | N,N-Diisopropylethylamine | Aldrich Chemical Co., Inc. |
| 6. | Dichloromethane | J.T. Baker Chemical Co. |
| 7. | EtOAc | EM Science |
| 8. | Hexanes | EM Science |
| 9. | Hydrochloric acid | J.T. Baker Chemical Co. |
| 10. | Sodium bicarbonate | J.T. Baker Chemical Co. |
| 11. | Magnesium sulfate | J.T. Baker Chemical Co. |
| 12. | Silica gel | E.M. Science |
| 13. | Trifluoroacetic acid | Aldrich Chemical Co., Inc. |
| 14. | *p*-Toluenesulfonic acid monohydrate | Kodak |
| 15. | *tert*-Butyl methyl ether | Aldrich Chemical Co., Inc. |
| 16. | Diethyl ether | Fisher Scientific |
| 17. | Ethyl dichlorophosphate | Aldrich Chemical Co., Inc. |
| 18. | Phenol | Aldrich Chemical Co., Inc. |
| 19. | Triethylamine | Aldrich Chemical Co., Inc. |
| 20. | 4-Dimethylaminopyridine | Reilly Industries |
| 21. | THF | Aldrich Chemical Co., Inc. |
| 22. | NaCl | Cargill |
| 23. | Sodium sulfate | Aldrich Chemical Co., Inc. |
| 24. | LiOH | Aldrich Chemical Co., Inc. |
| 25. | Hydrochloric acid, 0.985N | Aldrich Chemical Co., Inc. |
| 26. | $CH_3CN$ | Aldrich Chemical Co., Inc. |

2-(Guanin-7-yl)ethyl 2-hydroxyethyl sulfide (3)

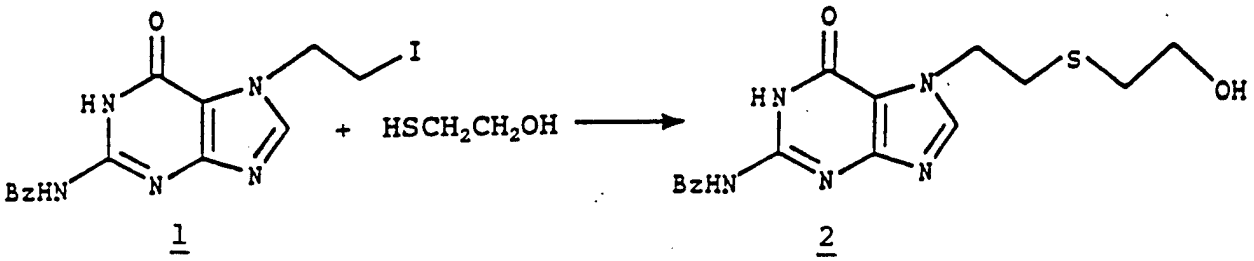
The target compound 3 was prepared by the following sequence of reactions. Intermediate 2 which is new to the Program will be transmitted to WRAIR.

Reaction Sequence:



15a. 2-(2-Benzamidoguanin-7-yl)ethyl 2-hydroxyethyl sulfide (2)

Reaction Sequence




1 2

Experimental

2-(Benzamido)-7-(2-iodoethyl)purin-6-one (1)

Please refer to p. 106, this report.

2-(2-Benzamidoguanin-7-yl)ethyl 2-hydroxyethyl sulfide (2)

To a solution of 2-mercaptoethanol (1.22 g, 15.6 mmol) in DMSO (30 mL) was added a 25% w/w solution of sodium methoxide in methanol (3.38 g soln= 0.845 g, 15.6 mmol). The mixture was stirred at room temperature for 30 minutes then compound 1 (6.4 g, 15.6 mmol) was added and quantitatively rinsed into the flask with DMSO (20 mL); a temperature rise of +4.5°C was observed. The mixture was stirred 30 min after which time TLC indicated some starting material 1 still remained. In a separate container were mixed 2-mercaptoethanol (0.55 mL, 7.8 mmol), DMSO (2 mL) and 25% sodium methoxide in methanol (1.69 g, 7.8 mmol), stirred 15 min then added to the reaction mixture. After stirring 40 min TLC indicated that most, but not all of the starting material 1 was gone. Again, mixed 2-mercaptoethanol (0.28 mL, 3.9 mmol), DMSO (1 mL) and 25% sodium methoxide in methanol (0.21 g, 3.9 mmol), stirred 15 min and added to the reaction mixture. After stirring 30 min, TLC indicated no starting

material 1 remained. The reaction mixture was concentrated to 5.30 g oil, redissolved in $CH_2Cl_2$:MeOH (9:1) (30 mL) and applied to a 250 g column of silica gel. The column was eluted with $CH_2Cl_2$:MeOH (9:1). Fractions containing the pure product (2) were concentrated to 2.13 g white solid. Fractions containing the product (2) plus some impurities were concentrated to a solid, triturated in $CH_2Cl_2$ filtered and dried to 2.88 g pure compound 2, to give a total of 5.01 g (13.9 mmol, 89.1%) compound 2. An additional 6.87 g compound 2 was obtained from a second reaction. A portion (2.7 g) will be tramsmitted to WRAIR (Lot No. NJ11-143-2), the remainder was used with the next reaction.

Anal.

| | | C | H | N | S |
|---|---|---|---|---|---|
| Calc'd for | $C_{16}H_{17}N_5O_3S$ | 53.47 | 4.77 | 19.49 | 8.92 |
| | Found | 53.25 | 4.84 | 19.59 | 9.06 |

Spectral Data

Infrared (Nujol)

Major bands: 3370, 3170, 3100, 3050, 1690, 1675, 1660, 1605, 1555, 1540, 1450, 1420, 1370, 1350, 1270, 1225, 1200, 1125, 1085, 1070, 1035, 895, 775, 685 $cm^{-1}$.

Ultraviolet (MeOH)

$\lambda_{max}$ 232 nm (log $\varepsilon$ 3.78); 278 nm (log $\varepsilon$ 3.72).

Nuclear Magnetic Resonance (DMSO-$d_6$)

$\delta$ 12.41 (s, 1, NH); 11.89 (s, 1, Ar NH); 8.28 (s, 1H, Ar); 8.10 (t, 2H, Bz); 7.72-7.70 (d, 1H, Bz); 7.61 (t, 2H, Bz); 4.84 (t, 1H, OH); 4.52 (t, 2H, $CH_2$); 3.61-3.57 (q, 2H, $CH_2$); 3.06 (t, 2H, $CH_2$); 2.64 (t, 2H, $CH_2$).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection ultraviolet light.

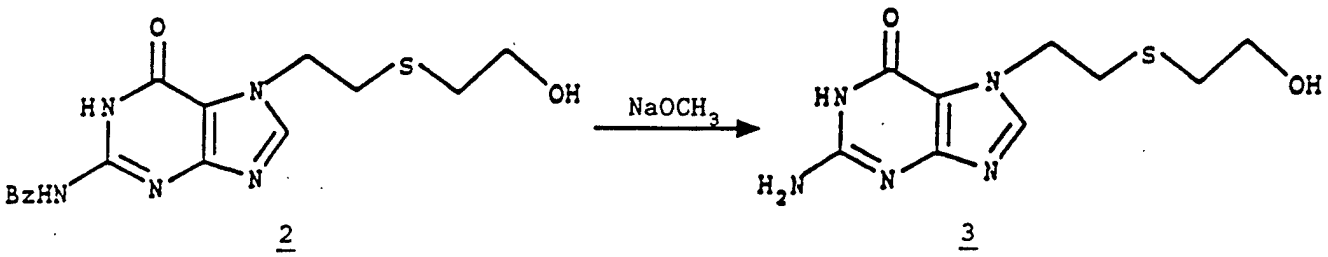
| | Eluent | Rf Value | Comment |
|---|---|---|---|
| 1. | $CH_2Cl_2$:MeOH (9:1) | 0.20 | Homogeneous |
| 2. | $CH_2Cl_2$:EtOH (4:1) | 0.57 | Homogeneous |
| 3. | EtOAc:MeOH (19:1) | 0.11 | Homogeneous |

Source of Material

| | | |
|---|---|---|
| 1. | Dichloromethane | J.T. Baker Chemical Co. |
| 2. | Methyl sulfoxide | Aldrich Chemical Co., Inc. |
| 3. | 2-Mercaptoethanol | Aldrich Chemical Co., Inc. |
| 4. | Methanol | J.T. Baker Chemical Co. |
| 5. | Sodium methoxide 25% w/w in methanol | Aldrich Chemical Co., Inc. |
| 6. | Silica gel | E.M. Science |

15b. 2-(Guanin-7-yl)ethyl 2-hydroxyethyl sulfide (3)

Reaction Sequence:




2 3

Experimental

2-(2-Benzamidoguanin-7-yl)ethyl 2-hydroxyethyl sulfide (2)

Please refer to the preceding synthesis, this report.

2-(Guanin-7-yl)ethyl 2-hydroxyethyl sulfide (3)

To a stirred suspension/solution of 2 (4.38 g, 12.2 mmol) in methanol (500 mL) was added a solution of 25% w/w sodium methoxide in methanol (12.5 g solution, 57.8 mmol). The stirred mixture was heated at reflux (66°C) for 5½ h, then allowed to cool while standing overnight. The absence of starting material 2 was confirmed by TLC and the mixture was concentrated to 9.90 g oily white solid. The solid was triturated in ether (approx. 70 mL) then collected. The recovered white powder was dissolved in water (20-25 mL) and filtered to remove any foreign matter. The aqueous solution (filtrate) was carefully neutralized with conc. HCl (approx. 4.3 mL) and then filtered. The collected white precipitate was dried in vacuo at 50°C to yield 3.08 g solid. The solid was suspended in $CH_2Cl_2$ with stirring then let stand overnight. The suspension was then filtered and the collected solid dried to 3.03 g compound 3 (11.9 mmol, 97.4%). An additional

1.29 g compound 3 was obtained from a scouting run. Both lots (4.32 g) were combined and stirred in 2 L boiling methanol for 20 min, then filtered while still hot. (The insoluble material was dried to 1.15 g off-white solid and consisted of compound 3 and some impurities). The clear filtrate was allowed to cool overnight. The crystals which had formed were collected by filtration and dried in a vacuum oven at 65°C to give 2.32 g pure compound 3, lot no. NJ11-147-4 a portion of which (50 mg) was submitted to WRAIR on April 10, 1996.

Anal.

| | | C | H | N | S |
|---|---|---|---|---|---|
| Calc'd for | $C_9H_{13}N_5O_2S$ | 42.35 | 5.13 | 27.43 | 12.56 |
| | Found | 42.43 | 5.09 | 27.32 | 12.46 |

Spectral Data

Infrared (Nujol)

Major bands: 3400, 3300, 2940, 2740, 1685, 1665, 1610, 1550, 1440, 1400, 1365, 1345, 1220, 1090, 1055, 1045, 870, 845, 770 cm-1.

Ultraviolet (MeOH)

$\lambda_{max}$ 210 nm (sh, log $\varepsilon$ 4.30); 232 nm (sh, 3.91); 287 nm (3.86).

Nuclear Magnetic Resonance ($DMSO-d_6$)

$\delta$ 10.76 (s, 1, NH); 7.96 (s, 1, CH at C-8); 6.16 (s, 2, $NH_2$ at C-2); 4.81 (s, 1, OH); 4.37 (t, 2, $CH_2$).

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection ultraviolet light.

| | Eluent | Rf Value | Comment |
|---|---|---|---|
| 1. | EtOAc:MeOH (2:1) | 0.22 | Trace impurity at Rf 0.88 |
| 2. | EtOH | 0.22 | Trace impurity at Rf 0.80 |
| 3. | $CH_2Cl_2$:MeOH (2:1) | 0.41 | Trace impurity at Rf 0.97 |

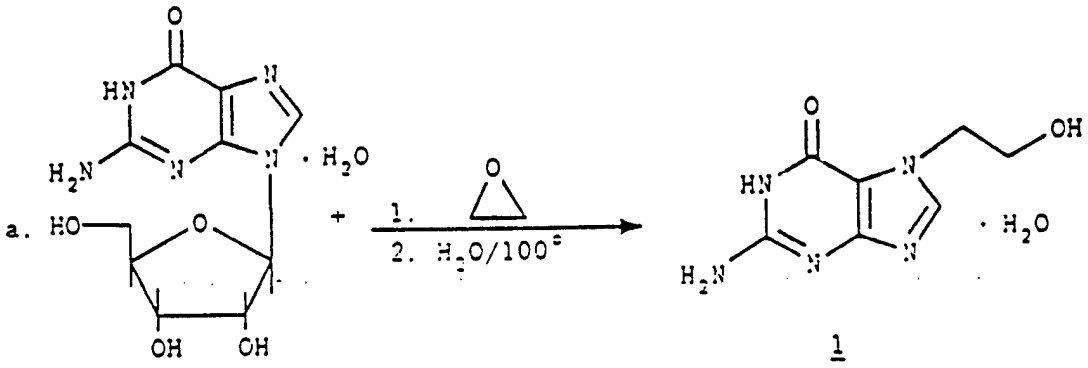
Source of Material

| | | |
|---|---|---|
| 1. | Dichloromethane | J.T. Baker Chemical Co. |
| 2. | Hydrochloric acid | J.T. Baker Chemical Co. |
| 3. | Diethyl ether | Fisher Scientific |
| 4. | Methanol | J.T. Baker Chemical Co. |
| 5. | Sodium methoxide in methanol | Aldrich Chemical Co., Inc. |

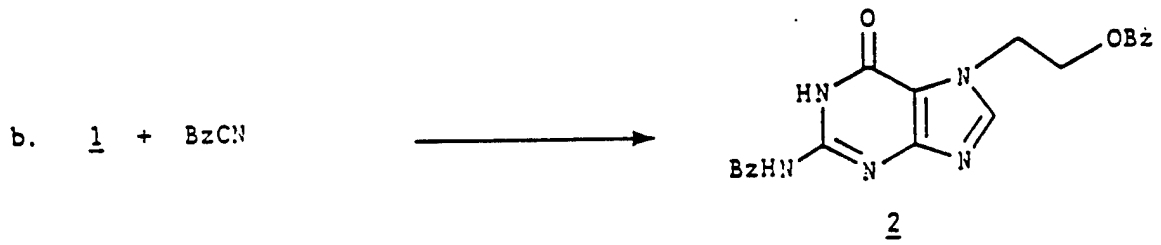
16. N-(2-Hydroxyethyl)-N-[2-(7-guaninyl)ethyl]methylamine (7)

The target compound 7 was prepared by the following sequence of reactions.

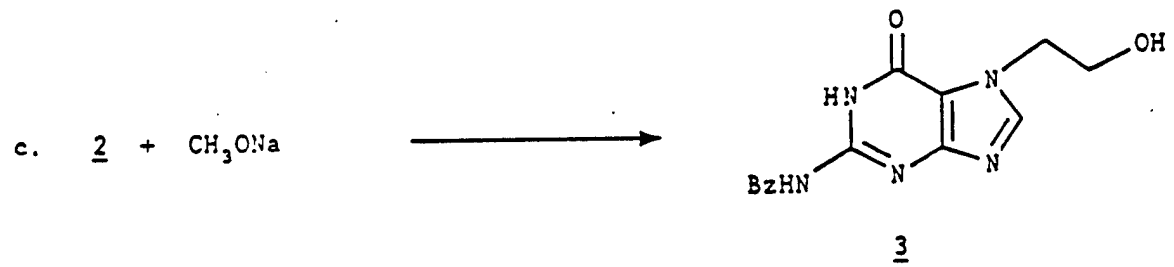
Reaction Sequence:



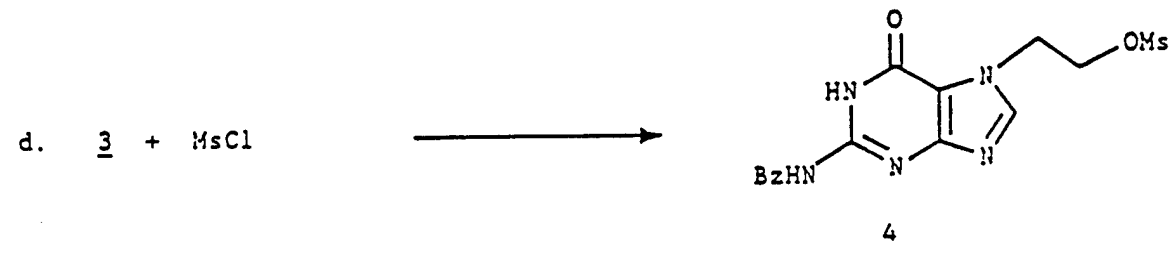

1




2

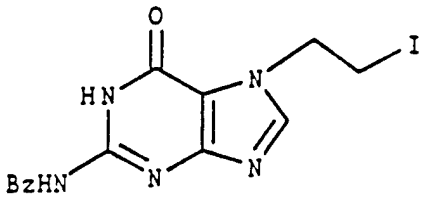



3



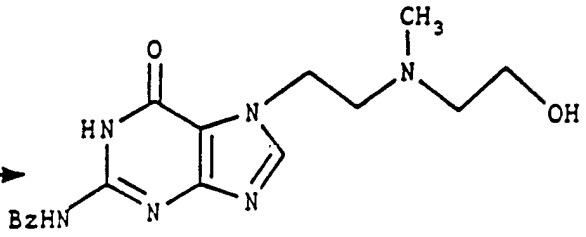

4

e. 4 + NaI ⟶



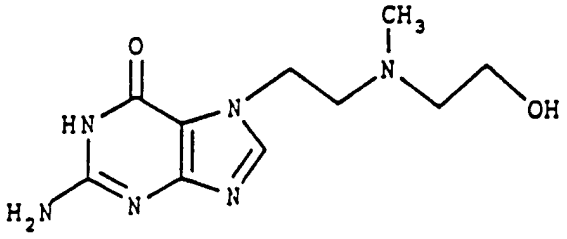
5

f. 5 + $HNCH_2CH_2OH$ (CH$_3$ on N) ⟶



6

g. 6 + $NaOCH_3$ ⟶



7

## Experimental

### 2-Amino-7-(2-hydroxyethyl)purin-6-one, monohydrate (1)

Guanosine hydrate (100.0 g, 353 mmol) was suspended in AcOH (2 L) and stirred, under argon atmosphere, for several minutes. Ethylene oxide (151.9 g, 3.45 mol) was added, and the suspension was stirred at RT for 16 h. A solution was obtained. Excess ethylene oxide and acetic acid was removed in vacuo, and the residue was dissolved in $H_2O$ (1000 mL), heated under reflux for 3 h, then cooled. The solid that separated was collected on a filter, washed with cold $H_2O$ (200 mL), then recrystallized from $H_2O$ (16 L) to give 1 as white

needles (63.9 g, 84.4%) mp >300°C; literature[1,2] mp >300°C and >325°C, respectively. The material was suitable for further transformation.

2-Benzamido-7-[2-(benzoyloxy)ethyl]purin-6-one (2)

Material 1 (58.9 g, 0.276 mol) was suspended in dry pyridine (1237 mL) containing benzoyl cyanide (119.0 g, 0.907 mol). After the addition of diisopropylethylamine (35.8 g, 0.277 mol) the mixture was stirred at 75°C for 2 3/4 h, then at room temperature overnight. The reaction mixture was quenched by addition of $H_2O$ (620 mL). The resulting precipitate was collected by filtration, washed with $CH_2Cl_2$ (3 x 190 mL) and dried in vacuo to give 91.0 g solid, mp 249-250°C; literature[1] mp 259-260°C. TLC (EtOAc:MeOH 9:1) showed one major spot corresponding to the product and one minor spot corresponding to the starting material. High field NMR confirmed both the identity of the product and the presence of starting material. Additional material (8.4 g) was obtained from another reaction. The material was suitable for further transformation.

2-Benzamido-7-(2-hydroxyethyl)purin-6-one (3)

A 25% solution of sodium methoxide in $CH_3OH$ (16.0 g, 74.0 mmol) was diluted with $CH_3OH$ (400 mL) then cooled to 0°C. To the solution was added 2 (10.0 g, 24.8 mmol), and the solution was stirred at 0°C for 1 h, under an argon atmosphere, at which time concentrated HCl was added until neutral to pH paper. The neutralized solution was stored at 0°C overnight and then filtered. The precipitate was washed with $CH_3OH$ (40 ml) then dried; yield, 6.1 g (82.2%); mp 222-225°C; literature[1] mp 234-235°C. An infrared spectrum matched previous spectra for this compound and TLC (EtOH:MeOH 9:1)

shows one major spot corresponding to the product and two minor spots representing trace impurities. Additional product (56.2 g) was obtained from two larger reactions. The material was suitable for further transformation.

2-Benzamido-7-[2-[(methanesulfonyl)oxy]ethyl]purin-6-one (4)

Material 3 (6.1 g, 20.4 mmol) was dissolved in pyridine (305 mL). Methanesulfonyl chloride (7.25 g, 63.3 mmol) was added, and the mixture was stirred, under an argon atmosphere, at room temperature overnight. The precipitate which had formed was collected by filtration, washed with a few mL of ethanol and dried to 7.5 g solid. The filtrate was diluted with $H_2O$ (600 mL), and the mixture was extracted with $CH_2Cl_2$:$CH_3OH$=9:1. The combined extracts were dried ($Na_2SO_4$) then concentrated in vacuo to 3.1 g solid. The two solids were combined and recrystallized from ethanol; three crops of material were collected, combined, and triturated in ethanol then dried to give 5.6 g (14.8 mmol, 72.8%). Additional product (65.4 g) was obtained from a scouting run and a larger reaction. The material was suitable for further transformation.

2-Benzamido-7-(2-iodoethyl)purin-6-one (5)

Compound 4 (10.0 g, 26.5 mmol) was added to NaI (55.0 g, 366.9 mmol) dissolved in 400 mL acetone. The suspension was stirred under reflux, in an argon atmosphere. After 4 h TLC of reaction showed the presence of starting material. Another 10.0 g of NaI was added and the reaction refluxed for a further 3 h, when the reaction was seen to be complete by TLC. The solvent was evaporated and the residue was partitioned between 10% $CH_3OH$ in $CH_2Cl_2$ (1.2 L) and water (1 L). The organic portion was separated and washed with $H_2O$ (500 mL),

dried over $Na_2SO_4$ and concentrated to dryness in vacuo to obtain 9.9 g (91.3%) of product. Additional product (4.1 g) was obtained from a scouting run. The material was suitable for further transformation.

N-(2-Hydroxyethyl)-N-[2-(2-benzamido-7-guaninyl)ethyl]-methylamine (6)

A stirred mixture of 2-benzamido-7-(2-iodoethyl)purin-6-one (5) (4.0 g, 9.77 mmol), methylaminoethanol (0.73 g, 9.77 mmol), and anhydrous potassium carbonate (1.35 g, 9.77 mmol) and 200 mL of acetonitrile was heated under reflux for 70 h. The solvent was removed on a rotary evaporator after cooling. The light brown reside was chromatographed on a column of silica gel (250 g), using $CH_2Cl_2$-MeOH (4:1) then $CH_2Cl_2$-MeOH (3:1) as the eluent. Fractions containing product were combined and concentrated to give 2.4 g of solid. Another 9.9 g of material of similar purity was obtained from a larger run. These were combined and rechromatographed on 1 kg $SiO_2$ with $CH_2Cl_2$-MeOH (9:1) as eluent to obtain 9.8 g of white solid. The material was suitable for further transformation.

N-(2-Hydroxyethyl)-N-[2-(7-guaninyl)ethyl]methylamine (7)

N-(2-Hydroxyethyl)-N-[2-(2-benzamido-7-guaninyl)ethyl]-methylamine 6 (2.0 g, 5.86 mmol) was suspended in 20 mL of MeOH and 6.0 mL of 25% sodium methoxide in MeOH was added. The reaction mixture was heated under reflux for 6 h, under argon atmosphere. The solvent was removed in vacuo to give a residue (3.5 g). The residue was applied on a column of silica gel (200 g), and eluted with $CH_2Cl_2$-MeOH (4:1) , $CH_2Cl_2$-MeOH (3:2). Fractions containing product were combined and evaporated to dryness to obtain 0.5 g product with 97.5% purity. This was combined with 1.64 g of material of similar

purity obtained from a larger run and chromatographed twice on silica gel with $CH_2Cl_2$-MeOH (4:1), $CH_2Cl_2$-MeOH (3:1) as eluent. Fractions containing product were combined and evaporated to dryness. The residue was dissolved in 25 mL $H_2O$ and treated with activated carbon, then crystallized from EtOH-$H_2O$ (1:1; 20 mL) to give 840 mg of pure product; mp 246-248°C (d). A portion (800 mg) was transmitted to WRAIR on May 23, 1996. (Lot No. NJ12-147-1).

Anal.

| | | C | H | N |
|---|---|---|---|---|
| Calc'd for | $C_{10}H_{16}N_6O_2$ | 47.61 | 6.39 | 33.31 |
| | Found | 47.69 | 6.41 | 33.25 |

Spectral Data

Infrared (KBr)

Major bands: 3280, 3130, 2860, 1670, 1550, 1535, 1465, 1380, 1270, 1215, 1090, 1025 $cm^{-1}$.

Ultraviolet (Ethanol)

$\lambda_{max}$ 238 nm (log $\varepsilon$ 3.80); 285 nm (3.87).

Nuclear Magnetic Resonance (DMSO-$d_6$)

$\delta$ 10.73 (s, 1, NH); 7.94 (s, 1, H at C-8); 6.12 (s, 2, $NH_2$); 4.32 (t, 1, OH); 4.28 (t, 2, J= 6.3 Hz, guanine -$\underline{CH_2}$); 3.40 (m, 2, J= 6.2, 5.6, 6.3 Hz, $\underline{CH_2}OH$); 2.77 (t, 2, J= 6.2 Hz guanine -$CH_2$-$\underline{CH_2}$); 2.47 (t,2, J= 6.4 Hz); $\underline{CH_2}CH_2OH$); 2.26 (s, 3, $CH_3$).

Assay by HPLC

| | |
|---|---|
| Column: | Zorbax C ODS, 4.6 x 250 mm. |
| Mobile Phase: | 20% $CH_3OH$/80% 0.05M Ammonium formate. |
| Flow rate: | 1 mL/min |
| Solvent Delivery System: | Waters 600E |
| Detector: | 285 nm |

Based on the HPLC trace the product NJ12-147-1 was pure.

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - UV light.

| | Eluent | Rf Value | Comment |
|---|---|---|---|
| 1. | $CH_2Cl_2$-MeOH (1:1) | 0.20 | elongated spot streaks |
| 2. | MeOH-$NH_4OH$ (10:1) | 0.80 | Homogeneous |

Source of Materials:

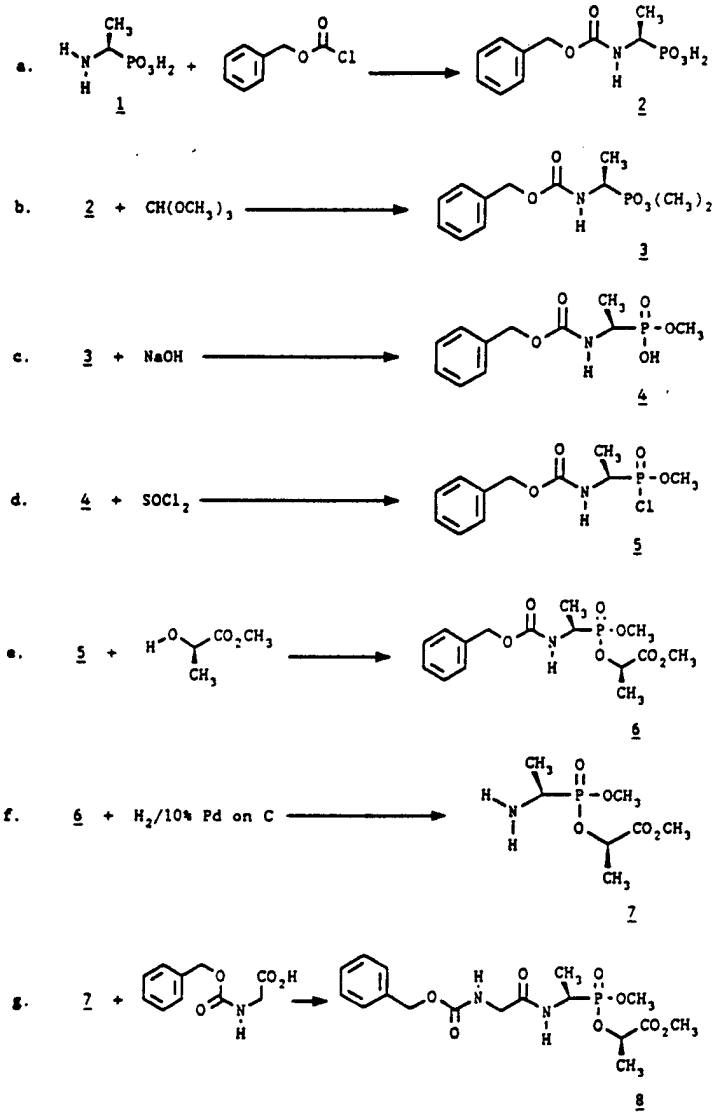
| | | |
|---|---|---|
| 1. | Guanosine hydrate | Aldrich Chemical Co., Inc. |
| 2. | AcOH | J.T. Baker Chemical Co. |
| 3. | Ethylene oxide | Eastman Kodak |
| 4. | 2-Amino-7-(2-hydroxy-ethyl)purin-6-one, monohydrate | Aldrich Chemical Co., Inc. |
| 5. | Pyridine | Aldrich Chemical Co., Inc. |
| 6. | Benzoyl cyanide | Aldrich Chemical Co., Inc. |
| 7. | DMAP | Aldrich Chemical Co., Inc. |
| 8. | $CHCl_3$ | Aldrich Chemical Co., Inc. |
| 9. | $CH_3CN$ | Aldrich Chemical Co., Inc. |
| 10. | 2-Benzamido-7-[2-benzoyloxy)ethyl]-purin-6-one | Starks Associates, Inc. |
| 11. | Sodium methoxide (25% in $CH_3OH$) | Aldrich Chemical Co., Inc. |
| 12. | HCl | J.T. Baker Chemical Co. |
| 13. | MeOH | J.T. Baker Chemical Co. |
| 14. | 2-Benzamido-7-(2-hydroxy-ethyl]purin-6-one | Starks Associates, Inc. |
| 15. | Methanesulfonyl chloride | Aldrich Chemical Co., Inc. |
| 16. | $CH_2Cl_2$ | Aldrich Chemical Co., Inc. |
| 17. | EtOH | US Industrial. |
| 18. | Banzamido-7-[2-[(methane-sulfonyl)oxy]ethyl]-purin-6-one | Starks Associates, Inc. |
| 19. | Acetone | J.T. Baker Chemical Co. |
| 20. | NaI | J.T. Baker Chemical Co. |

| | | |
|---|---|---|
| 22. | Methylaminoethanol | Aldrich Chemical Co., Inc. |
| 23. | Potassium carbonate | J.T. Baker Chemical Co. |
| 24. | Silica gel | EM Laboratories |
| 25. | N-(2-Hydroxyethyl)-N-[2-(2-benzamido-7-guaninyl)-ethyl]methylamine | Starks Associates, Inc. |

17. O-[[(1*R*)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(*R*)-lactic acid, methyl ester (8)

Intermediate 8 has been prepared by the following reaction sequence. 20 mg of 8 was transmitted to WRAIR on June 27, 1996 (Lot No. NJ20-146-2).

Reaction Sequence



## Experimental[19-22]

### [(1*R*)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (2)

To a stirred slurry of (*R*)-(-)-(1-aminoethyl)phosphonic-acid (1) (3.06 g, 24.5 mmol), sodium hydrogen carbonate (4.11 g, 48.9 mmol), sodium carbonate (5.19 g, 49.0 mmol), and 2N sodium hydroxide (25 mL, 50.0 mmol) at 5-10°C was added benzyl chloroformate (3.6 mL, 25.2 mmol) slowly by syringe. Two additional 3.6 mL portions of benzyl chloroformate were added at 1.0 h intervals, and then the mixture was stirred at room temperature for 20 h. To the white suspension was added enough 2N NaOH (26 mL) and $H_2O$ (100 mL) to completely dissolve the mixture. This aqueous solution was washed with $Et_2O$ (2 x 75 mL), acidified to pH 1-2 with conc. HCl, and then extracted with EtOAc (3 x 75 mL). The combined organic layer was washed with brine (75 mL), dried (4Å molecular sieves), filtered, and concentrated to give 2 (5.0 g, 79%) as a white foam which solidified. Additional reactions were performed to give a total of 17.5 g of 2.

### Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 10.0-9.60 (br m, 2H, -P(O<u>H</u>)$_2$); 7.26 (s, 5H, Ph-); 5.65 (br m, 1H, NH); 5.11-4.92 (m, 2H, Ph-<u>C</u>H$_2$); 4.10-3.90 (br m, 1H, -CH); 1.28-1.24 (dd, 3H, $CH_3$).

Dimethyl [(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]-phosphonate (3)

A solution of [(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (6.0 g, 23.2 mmol) in trimethyl orthoformate (185 mL) was heated to reflux for 48 h. After cooling, the solution was concentrated to give a residue, which was purified by silica gel chromatography (5% EtOH/$CH_2Cl_2$, 300 g $SiO_2$) to give 3 (3.2 g, 48%) as a colorless oil which solidified on standing at 0°C. Additional reactions were performed to give a total of 8.1 g of 3.

Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 7.35 (s, 5H, Ph-); 5.12 (s, 2H, Ph$\underline{CH_2}$-); 5.00-4.95 (br d, 1H, NH); 4.25-4.15 (br m, 1H, CH); 3.75-3.73 (2s, 6H, 2 x O$\underline{CH_3}$); 1.41-1.37 (dd, 3H, $CH_3$).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 28.25 (s, 1P).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

| Eluent | Rf Value | Comment |
|---|---|---|
| $CH_2Cl_2$-EtOH (19:1) | 0.32 | Homogeneous |

Methyl hydrogen[(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (4)

A solution of dimethyl[(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (2.7 g, 9.4 mmol) in $CH_3OH$ (11 mL) and 2N NaOH (7.26 mL, 14.5 mmol) was stirred at room temperature for 24 h. After dilution with $H_2O$ (30 mL), the solution was washed with $Et_2O$ (2 x 30 mL), acidified with 6N HCl to pH 1-2, and extracted with $CH_2Cl_2$ (4 x 75 mL). The combined $CH_2Cl_2$ was dried ($MgSO_4$), filtered, and concentrated to give a residue which was coevaporated with $Et_2O$ (2 x 20 mL) and then dried in vacuo to give 4 (2.28 g, 89%) as a white solid. Additional reactions were performed to give a total of 5.4 g of 4.

Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 10.1 (br, 1H, -P(OH)); 7.33 (s, 5H, Ph-);
5.80-5.20 (br, 1H, NH); 5.11 (s, 2H, Ph$CH_2$-);
3.90-4.16 (br, 1H, CH); 3.70 (d, 3H, -O$CH_3$);
1.36-1.32 (dd, 3H, $CH_3$).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 27.75 (s, 1P) (A small shoulder is also seen at $\delta$ 26.96, minor conformer).

FT - IR (KBr)

Major bands: 3283, 2643, 2301, 1689, 1543, 1453, 1374, 1302, 1260, 1231, 1159, 1114, 1047, 991, 810, 696, 549 $cm^{-1}$.

O-[[(1*R*)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]methoxyphosphinyl]-(*R*)-lactic acid, methyl ester (6)

A solution of methyl hydrogen[(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (2.7 g, 9.9 mmol) in $CH_2Cl_2$ (60 mL) was treated with 2M thionyl chloride in $CH_2Cl_2$ (5.54 mL, 11.1 mmol) and stirred for 4 h at room temperature. The solvent was removed and the residue was pumped dry in vacuo for 0.5 h to give the chloridate 5 as a yellow oil (2.9 g, 100%, crude product). This material was dissolved in $CH_2Cl_2$ (54 mL), chilled to 0-5°C under an argon atmosphere and a solution of methyl (*R*)-(+)-lactate (1.027 mL, 10.75 mmol, 98%) and $Et_3N$ (1.76 mL, 12.6 mmol) in $CH_2Cl_2$ (27 mL) was added dropwise. Stirred at 5°C for 15 min then stirred at RT for 64 h. The solution was concentrated to give a residue which was dissolved in EtOAc (100 mL) and washed with $H_2O$ (50 mL), sat. $NaHCO_3$ (50 mL), 3M HCl (50 mL), and brine (75 mL), then dried ($MgSO_4$), filtered and concentrated to give the crude product (3.0 g, 85%) as a tan oil, which was purified by silica gel chromatography (85:15 EtOAc:hexanes, 80 g $SiO_2$) to give pure 6 (2.6 g, 73%), as a colorless oil. Additional reactions were performed to give a total of 4.7 g of 6 (2.9 g of 6 is currently available for conversion to 8).

Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 7.36-7.30 (m, 5H, Ph-); 5.45 (br d, 0.5H, NH); 5.14-4.93 (3m, 3.5H, Ph$\underline{CH_2}$ + $\underline{CH}$ + 0.5 $\underline{NH}$); 4.35-4.10 (2m, 1H, $\underline{CH}$); 3.83-3.75 (4s, 6H, -$CO_2\underline{CH_3}$ and P-O$\underline{CH_3}$); 1.57-1.50 (2d, 3H, -OCH($\underline{CH_3}$)CO-); 1.43-1.37 (dddd, 3H, -NCH($\underline{CH_3}$)P).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 27.78 (s); 26.73 (s).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc-hexanes (85:15) | 0.36 | Homogeneous |

O-[[(1*R*)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(*R*)-lactic acid methyl ester (8)

A solution of O-[[(1*R*)-N-(phenylmethoxycarbonyl)-1-amino-ethyl]methoxyphosphinyl]-(*R*)-lactic acid, methyl ester (1.0 g, 2.78 mmol) in EtOAc (50 mL) was hydrogenated at 15 psi. for 1.0 h over 10% palladium on carbon (0.5 g) (Parr hydrogenation apparatus). The suspension was filtered through a celite pad; the pad was washed with EtOAc (2 x 50 mL) and the combined filtrates were concentrated to give crude 7 as a colorless oil, which was used immediately in the next step below.

To a solution of carbobenzyloxyglycine (700 mg, 3.35 mmol) in THF (20 mL) at 0°C under argon was added 4-methylmorpholine (368 $\mu$l, 3.35 mmol) followed by isobutyl chloroformate (435 $\mu$L, 3.35 mmol). After stirring at 0°C for 15 min, additional 4-methylmorpholine (368 $\mu$l, 3.35 mmol) was added followed by a solution of the crude amine 7 (prepared above) in THF (15 mL). After stirring at 0°C for 3 h, the reaction was quenched by the addition of sat. $NH_4Cl$ (3 mL). The suspension was filtered and the funnel was washed with THF (2 x 25 mL). The combined filtrate was concentrated and the

residue dissolved in $CH_2Cl_2$ (100 mL) and washed with 1.0M HCl (2 x 50 mL), sat. $NaHCO_3$ (2 x 50 mL), and brine (100 mL). The $CH_2Cl_2$ layer was dried ($MgSO_4$), filtered and concentrated to give the crude product (1.1 g, 95%), which was purified on a silica gel column (97:3 EtOAc:EtOH, 60 g $SiO_2$) to give pure 8 (0.9 g, 78%) as a colorless viscous oil. A portion (20 mg) was transmitted to WRAIR on June 27, 1996 (Lot No. NJ20-146-2).

Anal.

| | C | H | N |
|---|---|---|---|
| Calc'd. for $C_{17}H_{25}N_2O_8P$ | 49.04 | 6.05 | 6.73 |
| Found | 48.92 | 6.03 | 6.67 |

Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 7.35 (s, 5H, Ph-); 6.84 and 6.45 (2 br d, 1H, NH); 5.51 and 5.46 (2m, 1H, NH); 5.13 (s, 2H, $PhCH_2$-); 5.01 and 4.94 (2m, 1H, $-OCH(CH_3)CO_2$-); 4.60 and 4.53 (2m, 1H, $-NHCH(CH_3)P$-); 3.99-3.85 (m, 2H, $-NHCH_2CO$-); 3.83-3.73 (m, 6H, $-CO_2CH_3$ and $P-OCH_3$); 1.56-1.52 (2d, 3H, $-OCH(CH_3)CO$-); 1.39-1.34 (m, 3H, $-NHCH(CH_3)P$-).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 27.83 (s); 26.38 (s).

FT - IR (thin film on KBr plate)

Major bands: 3288, 3065, 2957, 2855, 1722, 1680, 1537, 1454, 1379, 1309, 1232, 1101, 1043, 996, 859, 827, 744, 701, 558, 460 $cm^{-1}$.

Mass Spectrum

Method of Ionization = DEI (low temp)

Calc'd for $C_{17}H_{25}N_2PO_8$ = 416.1

Found: 416, $(m)^+$.

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc-EtOH (97:3) | 0.26 | Homogeneous |

Source of Materials

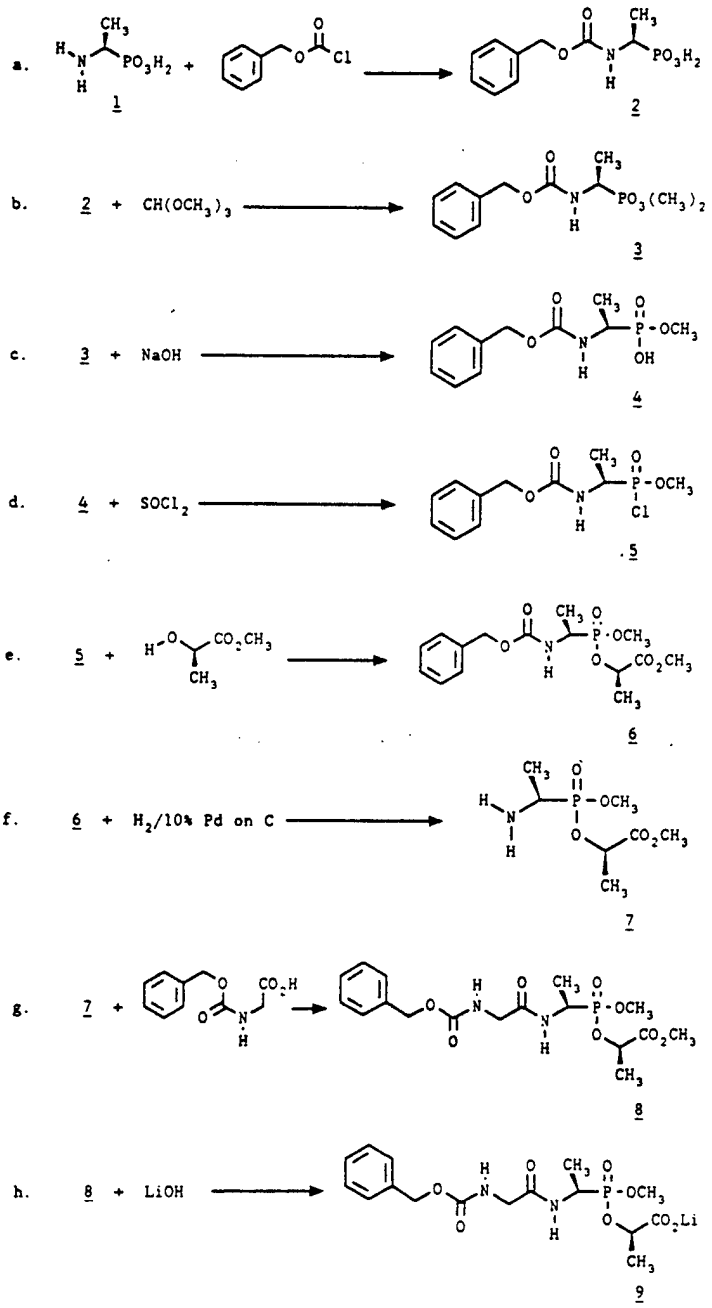
| | | |
|---|---|---|
| 1. | (*R*)-(-)-(1-Aminoethyl)-phosphonic acid | Aldrich Chemical Co., Inc. |
| 2. | Sodium hydrogen carbonate | J.T. Baker Chemical Co. |
| 3. | Sodium carbonate | J.T. Baker Chemical Co. |
| 4. | Sodium hydroxide | J.T. Baker Chemical Co. |
| 5. | Benzyl chloroformate | Aldrich Chemical Co., Inc. |
| 6. | Ether | Fisher Scientific |
| 7. | Hydrochloric acid | J.T. Baker Chemical Co. |
| 8. | Ethyl acetate | E.M. Science |
| 9. | Molecular sieves (4Å) | Aldrich Chemical Co., Inc. |
| 10. | Trimethyl orthoformate | Fluka |
| 11. | Silica gel | E.M. Science |
| 12. | Ethanol | US Industrial Chem. Corp. |
| 13. | Dichloromethane | J.T. Baker Chemical Co. |
| 14. | Methanol | J.T. Baker Chemical Co. |

| | | |
|---|---|---|
| 15. | Thionyl chloride (2M in $CH_2Cl_2$) | Aldrich Chemical Co., Inc. |
| 16. | Methyl (*R*)-(+)-lactate | Aldrich Chemical Co., Inc. |
| 17. | Triethylamine | Aldrich Chemical Co., Inc. |
| 18. | Magnesium sulfate | J.T. Baker Chemical Co. |
| 19. | Hexanes | J.T. Baker Chemical Co. |
| 20. | 10% Palladium on carbon | Aldrich Chemical Co., Inc. |
| 21. | Hydrogen (gas) | Matheson |
| 22. | Celite | Manville |
| 23. | Carbobenzyloxyglycine | Aldrich Chemical Co., Inc. |
| 24. | Tetrahydrofuran | Aldrich Chemical Co., Inc. |
| 25. | 4-Methylmorpholine | Aldrich Chemical Co., Inc. |
| 26. | Isobutyl chloroformate | Aldrich Chemical Co., Inc. |
| 27. | Ammonium chloride | Aldrich Chemical Co., Inc. |

18. O-[[(1*R*)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(*R*)-lactic acid, lithium salt (9)

Compound 9 has been prepared by the following reaction sequence. 20 mg of 9 was transmitted to WRAIR on June 27, 1996 (Lot No. NJ22-21-1).

Reaction Sequence



## Experimental[19-22]

### [(1*R*)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (2)

To a stirred slurry of (*R*)-(-)-(1-aminoethyl)phosphonic-acid (1) (3.06 g, 24.5 mmol), sodium hydrogen carbonate (4.11 g, 48.9 mmol), sodium carbonate (5.19 g, 49.0 mmol), and 2N sodium hydroxide (25 mL, 50.0 mmol) at 5-10°C was added benzyl chloroformate (3.6 mL, 25.2 mmol) slowly by syringe. Two additional 3.6 mL portions of benzyl chloroformate were added at 1.0 h intervals, and then the mixture was stirred at room temperature for 20 h. To the white suspension was added enough 2N NaOH (26 mL) and $H_2O$ (100 mL) to completely dissolve the mixture. This aqueous solution was washed with $Et_2O$ (2 x 75 mL), acidified to pH 1-2 with conc. HCl, and then extracted with EtOAc (3 x 75 mL). The combined organic layer was washed with brine (75 mL), dried (4Å molecular sieves), filtered, and concentrated to give 2 (5.0 g, 79%) as a white foam which solidified. Additional reactions were performed to give a total of 17.5 g of 2.

### Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 10.0-9.60 (br m, 2H, $-P(O\underline{H})_2$); 7.26 (s, 5H, Ph-); 5.65 (br m, 1H, NH); 5.11-4.92 (m, 2H, Ph-$\underline{CH}_2$); 4.10-3.90 (br m, 1H, -CH); 1.28-1.24 (dd, 3H, dd, 3H, $CH_3$).

Dimethyl [(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]-phosphonate (3)

A solution of [(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (6.0 g, 23.2 mmol) in trimethyl orthoformate (185 mL) was heated to reflux for 48 h. After cooling, the solution was concentrated to give a residue, which was purified by silica gel chromatography (5% EtOH/$CH_2Cl_2$, 300 g $SiO_2$) to give 3 (3.2 g, 48%) as a colorless oil which solidified on standing at 0°C. Additional reactions were performed to give a total of 8.1 g, of 3.

Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 7.35 (s, 5H, Ph-); 5.12 (s, 2H, Ph$\underline{CH_2}$-); 5.00-4.95 (br d, 1H, NH); 4.25-4.15 (br m, 1H, CH); 3.75-3.73 (2s, 6H, 2 x O$\underline{CH_3}$); 1.41-1.37 (dd, 3H, $CH_3$).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 28.25 (s, 1P).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

| Eluent | Rf Value | Comment |
|---|---|---|
| $CH_2Cl_2$-EtOH (19:1) | 0.32 | Homogeneous |

Methyl hydrogen[(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (4)

A solution of dimethyl [(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (2.7 g, 9.4 mmol) in $CH_3OH$ (11 mL) and 2N NaOH (7.26 mL, 14.5 mmol) was stirred at room temperature for 24 h. After dilution with $H_2O$ (30 mL), the solution was washed with $Et_2O$ (2 x 30 mL), acidified with 6N HCl to pH 1-2, and extracted with $CH_2Cl_2$ (4 x 75 mL). The combined $CH_2Cl_2$ was dried ($MgSO_4$), filtered, and concentrated to give a residue which was coevaporated with $Et_2O$ (2 x 20 mL) and then dried in vacuo to give 4 (2.28 g, 89%) as a white solid. Additional reactions were performed to give a total of 5.4 g of 4.

Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 10.1 (br, 1H, -P(OH)); 7.33 (s, 5H, Ph-); 5.80-5.20 (br, 1H, NH); 5.11 (s, 2H, Ph$CH_2$-); 3.90-4.16 (br, 1H, CH); 3.70 (d, 3H, -O$CH_3$); 1.36-1.32 (dd, 3H, $CH_3$).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 27.75 (s, 1P) (A small shoulder is also seen at $\delta$ 26.96, minor conformer).

FT - IR (KBr)

Major bands: 3283, 2643, 2301, 1689, 1543, 1453, 1374, 1302, 1260, 1231, 1159, 1114, 1047, 991, 810, 696, 549 $cm^{-1}$.

O-[[(1*R*)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]methoxyphosphinyl]-(*R*)-lactic acid, methyl ester (6)

A solution of methyl hydrogen[(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (2.7 g, 9.9 mmol) in $CH_2Cl_2$ (60 mL) was treated with 2M thionyl chloride in $CH_2Cl_2$ (5.54 mL, 11.1 mmol) and stirred for 4 h at room temperature. The solvent was removed and the residue was pumped dry in vacuo for 0.5 h to give the chloridate 5 as a yellow oil (2.9 g, 100%, crude product). This material was dissolved in $CH_2Cl_2$ (54 mL), chilled to 0-5°C under an argon atmosphere and a solution of methyl (*R*)-(+)-lactate (1.027 mL, 10.75 mmol, 98%) and $Et_3N$ (1.76 mL, 12.6 mmol) in $CH_2Cl_2$ (27 mL) was added dropwise. Stirred at 5°C for 15 min then stirred at RT for 64 h. The solution was concentrated to give a residue which was dissolved in EtOAc (100 mL) and washed with $H_2O$ (50 mL), sat. $NaHCO_3$ (50 mL), 3M HCl (50 mL), and brine (75 mL), then dried ($MgSO_4$), filtered and concentrated to give the crude product (3.0 g, 85%) as a tan oil, which was purified by silica gel chromatography (85:15 EtOAc:hexanes, 80 g $SiO_2$) to give pure 6 (2.6 g, 73%), as a colorless oil. Additional reactions were performed to give a total of 4.7 g of 6 (2.9 g of 6 is currently available for conversion to 8).

Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 7.36-7.30 (m, 5H, Ph-); 5.45 (br d, 0.5H, NH); 5.14-4.93 (3m, 3.5H, Ph$\underline{CH_2}$ + $\underline{CH}$ + 0.5 $\underline{NH}$); 4.35-4.10 (2m, 1H, $\underline{CH}$); 3.83-3.75 (4s, 6H, -$CO_2\underline{CH_3}$ and P-O$\underline{CH_3}$); 1.57-1.50 (2d, 3H, -OCH($\underline{CH_3}$)CO-); 1.43-1.37 (dddd, 3H, -NCH($\underline{CH_3}$)P).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 27.78 (s); 26.73 (s).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc-hexanes (85:15) | 0.36 | Homogeneous |

O-[[(1*R*)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(*R*)-lactic acid methyl ester (8)

A solution of O-[[(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]methoxyphosphinyl]-(*R*)-lactic acid methyl ester (1.0 g, 2.78 mmol) in EtOAc (50 mL) was hydrogenated at 15 psi. for 1.0 h over 10% palladium on carbon (0.5 g) (Parr hydrogenation apparatus). The suspension was filtered through a celite pad; the pad was washed with EtOAc (2 x 50 mL) and the combined filtrates were concentrated to give crude 7 as a colorless oil, which was used immediately in the next step below.

To a solution of carbobenzyloxyglycine (700 mg, 3.35 mmol) in THF (20 mL) at 0°C under argon was added 4-methylmorpholine (368 $\mu$l, 3.35 mmol) followed by isobutyl chloroformate (435 $\mu$L, 3.35 mmol). After stirring at 0°C for 15 min, additional 4-methylmorpholine (368 $\mu$l, 3.35 mmol) was added followed by a solution of the crude amine 7 (prepared above) in THF (15 mL). After stirring at 0°C for 3 h, the reaction was quenched by the addition of sat. $NH_4Cl$ (3 mL). The suspension was filtered and the funnel was washed with THF (2 x 25 mL). The combined filtrate was concentrated and the residue dissolved in $CH_2Cl_2$ (100 mL) and washed with 1.0M HCl

(2 x 50 mL), sat. $NaHCO_3$ (2 x 50 mL), and brine (100 mL). The $CH_2Cl_2$ layer was dried ($MgSO_4$), filtered and concentrated to give the crude product (1.1 g, 95%), which was purified on a silica gel column (97:3 EtOAc:EtOH, 60 g $SiO_2$) to give pure 8 (0.9 g, 78%) as a colorless viscous oil.

Anal.

| | C | H | N |
|---|---|---|---|
| Calc'd. for $C_{17}H_{25}N_2O_8P$ | 49.04 | 6.05 | 6.73 |
| Found | 48.92 | 6.03 | 6.67 |

Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 7.35 (s, 5H, Ph-); 6.84 and 6.45 (2 br d, 1H, NH); 5.51 and 5.46 (2m, 1H, NH); 5.13 (s, 2H, Ph$\underline{CH_2}$-); 5.01 and 4.94 (2m, 1H, -OC$\underline{H}$($CH_3$)$CO_2$-); 4.60 and 4.53 (2m, 1H, -NH$\underline{CH}$($CH_3$)P-); 3.99-3.85 (m, 2H,-NH$\underline{CH_2}$CO-); 3.83-3.73 (m, 6H, -$CO_2\underline{CH_3}$ and P-O$\underline{CH_3}$); 1.56-1.52 (2d, 3H, -OCH($\underline{CH_3}$)CO-); 1.39-1.34 (m, 3H, -NHCH($\underline{CH_3}$)P-).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 27.83 (s); 26.38 (s).

FT - IR (thin film on KBr plate)

Major bands: 3288, 3065, 2957, 2855, 1722, 1680, 1537, 1454, 1379, 1309, 1232, 1101, 1043, 996, 859, 827, 744, 701, 558, 460 $cm^{-1}$.

Mass Spectrum

Method of Ionization = DEI (low temp)

Calc'd for $C_{17}H_{25}N_2PO_8$ = 416.1

Found: 416, $(m)^+$.

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc-EtOH (97:3) | 0.26 | Homogeneous |

O-[[(1*R*)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(*R*)-lactic acid, lithium salt (9)

To a solution of O-[[(1*R*)-N-[N-(phenylmethoxycarbonyl)-glycyl]-1-aminoethyl]methoxyphosphinyl]-(*R*)-lactic acid methyl ester (78 mg, 0.187 mmol) in THF (2 mL) and deionized $H_2O$ (1 mL) was added a solution of $LiOH \cdot H_2O$ in deionized $H_2O$ (1.5M, 125 $\mu$L, 0.203 mmol). After stirring at room temperature for 45 min., the mixture was concentrated to dryness and coevaporated with $CH_3CN$ (3 x 10 mL) to give lot no. NJ22-21-1 as a white powder (70 mg, 92%). A portion (20 mg) was transmitted to WRAIR on June 27, 1996 (Lot No. NJ22-21-1).

Anal.

| | | C | H |
|---|---|---|---|
| Calc'd. for $C_{16}H_{22}LiN_2O_8P \cdot 0.8LiOH \cdot 0.35H_2O$ | | 44.31 | 5.46 |
| | Found | 44.35 | 5.44 |
| | | 44.33 | 5.49 |

Spectral Data

$^{1}$H Nuclear Magnetic Resonance ($D_2O$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 7.31-7.29 (m, 5H, Ph-); 5.02 (s, 2H, Ph$\underline{CH_2}$-); 4.36 (m, 1H, -O$\underline{CH}$($CH_3$)$CO_2$-); 3.73 (s, 2H, -NH$\underline{CH_2}$CO-); 3.70-3.54 (m, 4H, -NH$\underline{CH}$($CH_3$)P- and P-O$\underline{CH_3}$); 1.36-1.32 (m, 3H, -OCH($\underline{CH_3}$)CO-); 1.25-1.22 (m, 2.2H, -NHCH($\underline{CH_3}$)P-, major conformer); 1.14 (m, 0.8H, -NHCH($\underline{CH_3}$)P-, minor conformer).

$^{31}$P Nuclear Magnetic Resonance ($D_2O$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 27.96 (s); 27.49 (s).

Mass Spectrum

Method of Ionization = Electrospray (negative)

Calc'd for $C_{16}H_{22}N_2O_8P$ = 402.12 (free acid)

Found: 401.4 $(m-H)^-$.

Source of Materials

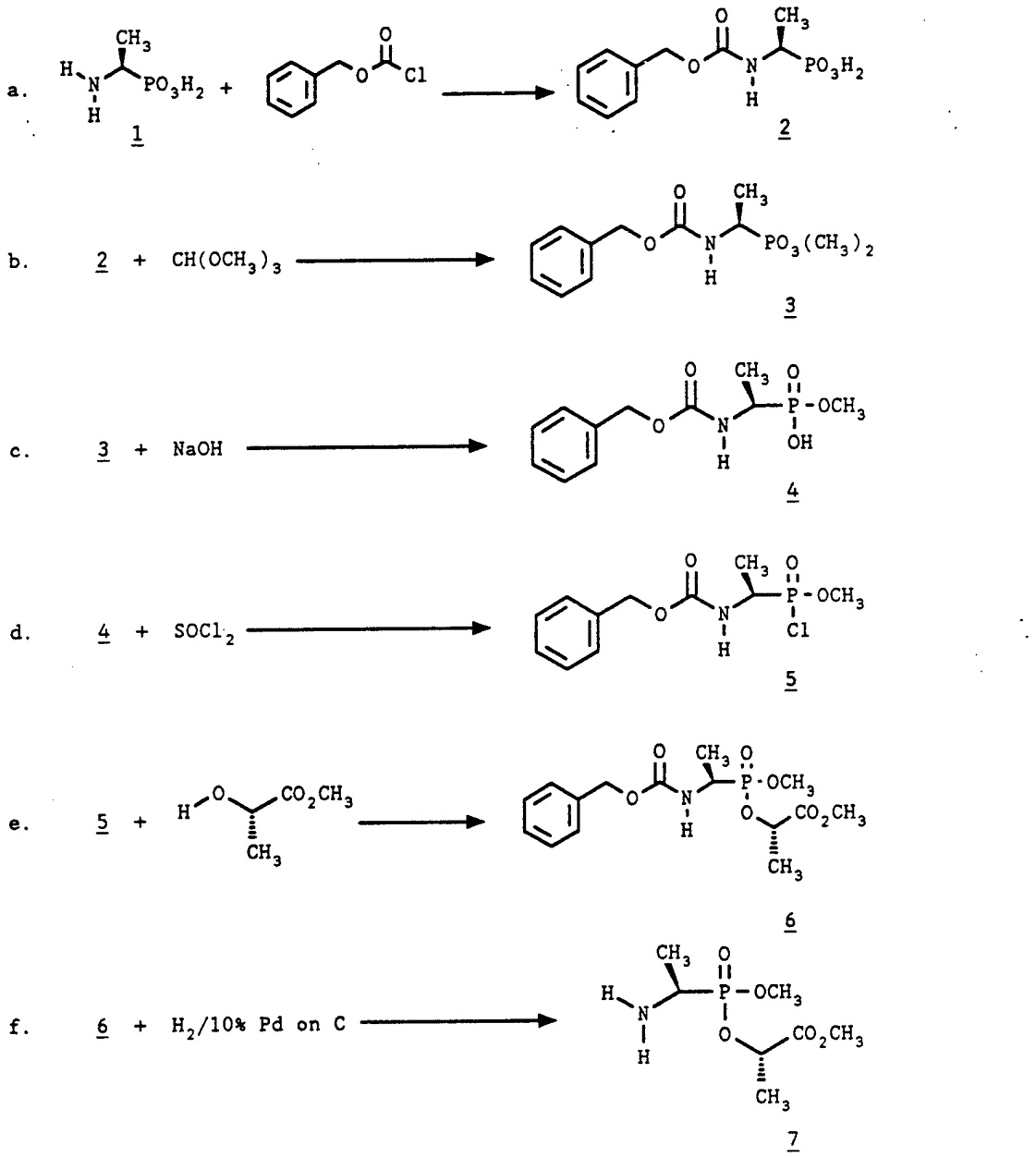
| | | |
|---|---|---|
| 1. | (*R*)-(-)-(1-Aminoethyl)-phosphonic acid | Aldrich Chemical Co., Inc. |
| 2. | Sodium hydrogen carbonate | J.T. Baker Chemical Co. |
| 3. | Sodium carbonate | J.T. Baker Chemical Co. |
| 4. | Sodium hydroxide | J.T. Baker Chemical Co. |
| 5. | Benzyl chloroformate | Aldrich Chemical Co., Inc. |
| 6. | Ether | Fisher Scientific |
| 7. | Hydrochloric acid | J.T. Baker Chemical Co. |
| 8. | Ethyl acetate | E.M. Science |
| 9. | Molecular sieves (4Å) | Aldrich Chemical Co., Inc. |
| 10. | Trimethyl orthoformate | Fluka |

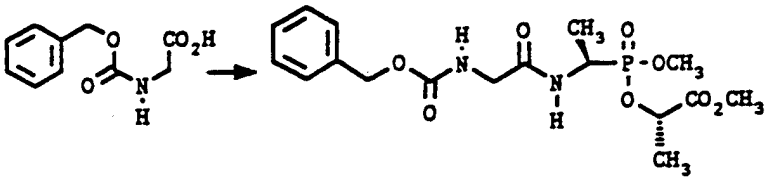
| | | |
|---|---|---|
| 11. | Silica gel | E.M. Science |
| 12. | Ethanol | US Industrial Chem. Corp. |
| 13. | Dichloromethane | J.T. Baker Chemical Co. |
| 14. | Methanol | J.T. Baker Chemical Co. |
| 15. | Thionyl chloride (2M in $CH_2Cl_2$) | Aldrich Chemical Co., Inc. |
| 16. | Methyl (*R*)-(+)-lactate | Aldrich Chemical Co., Inc. |
| 17. | Triethylamine | Aldrich Chemical Co., Inc. |
| 18. | Magnesium sulfate | J.T. Baker Chemical Co. |
| 19. | Hexanes | J.T. Baker Chemical Co. |
| 20. | 10% Palladium on carbon | Aldrich Chemical Co., Inc. |
| 21. | Hydrogen (gas) | Matheson |
| 22. | Celite | Manville |
| 23. | Carbobenzyloxyglycine | Aldrich Chemical Co., Inc. |
| 24. | Tetrahydrofuran | Aldrich Chemical Co., Inc. |
| 25. | 4-Methylmorpholine | Aldrich Chemical Co., Inc. |
| 26. | Isobutyl chloroformate | Aldrich Chemical Co., Inc. |
| 27. | Ammonium chloride | Aldrich Chemical Co., Inc. |
| 28. | Lithium hydroxide monohydrate | Aldrich Chemical Co., Inc. |
| 29. | Acetonitrile | Aldrich Chemical Co., Inc. |

19a. O-[[(*L*)-1-[[N-(Phenylmethoxycarbonyl)glycylamino]ethyl]-hydroxyphosphinyloxy]-*L*-lactic acid, dilithium salt (11)

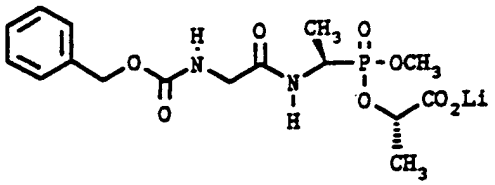
Compound 11 has been prepared by the following reaction sequence. 30 mg of 11 was transmitted to WRAIR on July 23, 1996 (Lot No. NJ22-52-1).

Reaction Sequence

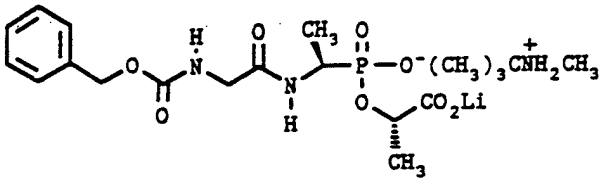


g. 7 + 

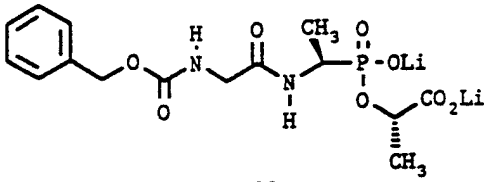
8

h. 8 + LiOH ⟶ 

9

i. 9 + tert-butylamine ⟶ 

10

j. 10 + AG50W-X8($Li^+$) ⟶ 

11

## Experimental[19-22]

### [(1*R*)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (2)

To a stirred slurry of (*R*)-(-)-(1-aminoethyl)phosphonic-acid (1) (6.07 g, 48.5 mmol), sodium hydrogen carbonate (8.16 g, 97.1 mmol), sodium carbonate (10.3 g, 97.2 mmol), and 2N sodium hydroxide (48.8 mL, 97.6 mmol) at 5-10°C was added benzyl chloroformate (7.2 mL, 50.4 mmol) slowly by syringe. Two additional 7.2 mL portions of benzyl chloroformate were added at 1.0 h intervals, and then the mixture was stirred at room temperature for 20 h. To the white suspension was added enough 2N NaOH (15 mL) and $H_2O$ (150 mL) to completely dissolve the mixture. This aqueous solution was washed with $Et_2O$ (2 x 100 mL), acidified to pH 1-2 with conc. HCl, and then extracted with EtOAc (3 x 120 mL). The combined organic layer was washed with brine (120 mL), dried (4Å molecular sieves), filtered, and concentrated to give 2 (11.3 g, 90%) as a white foam which solidified. An additional reaction was performed to give a total of 20.0 g of 2.

### Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 10.0-9.60 (br m, 2H, $-P(O\underline{H})_2$); 7.26 (s, 5H, Ph-); 5.65 (br m, 1H, NH); 5.11-4.92 (m, 2H, Ph-$\underline{CH_2}$); 4.10-3.90 (br m, 1H, -CH); 1.28-1.24 (dd, 3H, dd, 3H, $CH_3$).

Dimethyl [(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]-phosphonate (3)

A solution of [(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (8.7 g, 33.6 mmol) in trimethyl orthoformate (265 mL) was heated to reflux for 48 h. After cooling, the solution was concentrated to give a residue, which was purified by silica gel chromatography (5% EtOH/$CH_2Cl_2$, 367 g $SiO_2$) to give 3 (5.1 g, 53%) as a colorless oil which solidified on standing at 0°C. An additional reaction was performed to give a total of 10.1 g, of 3.

Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 7.35 (s, 5H, Ph-); 5.12 (s, 2H, Ph$\underline{CH_2}$-); 5.00-4.95 (br d, 1H, NH); 4.25-4.15 (br m, 1H, CH); 3.75-3.73 (2s, 6H, 2 x O$\underline{CH_3}$); 1.41-1.37 (dd, 3H, $CH_3$).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 28.24 (s, 1P).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

| Eluent | Rf Value | Comment |
|---|---|---|
| $CH_2Cl_2$-EtOH (19:1) | 0.32 | Homogeneous |

Methyl hydrogen[(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (4)

A solution of dimethyl [(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (5.0 g, 17.4 mmol) in $CH_3OH$ (20.5 mL) and 2N NaOH (13.5 mL, 27.0 mmol) was stirred at room temperature for 24 h. After dilution with $H_2O$ (50 mL), the solution was washed with $Et_2O$ (2 x 60 mL), acidified with 6N HCl to pH 1-2, and extracted with $CH_2Cl_2$ (4 x 100 mL). The combined $CH_2Cl_2$ was washed with brine (100 mL), dried ($Na_2SO_4$), filtered, and concentrated to give a residue which was coevaporated with $Et_2O$ (20 mL) and then dried in vacuo to give 4 (4.3 g, 90%) as a white solid, mp 119-122°C. An additional reaction was performed to give a total of 9.0 g of 4.

Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 7.33 (s, 5H, Ph-); 6.89 (m, 1H, -P(OH)); 5.73 (m, 0.2H, NH, minor conformer); 5.27 (m, 0.8H, NH, major conformer); 5.11 (s, 2H, Ph$CH_2$-); 4.16 (m, 0.8H, CH, major conformer); 4.02 (m, 0.2H, CH, minor conformer); 3.70 (d, 3H, -O$CH_3$); 1.36-132 (dd, 2.4H, J= 7.0, 16.7, $CH_3$, major conformer); 1.25 (m, 0.6H, $CH_3$, minor conformer).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 28.63 (s, ~80%, major conformer); 27.89 (br, ~20%, minor conformer).

FT - IR (KBr)

Major bands: 3283, 2643, 2301, 1689, 1543, 1453, 1374, 1302, 1260, 1231, 1159, 1114, 1047, 991, 810, 696, 549 $cm^{-1}$.

O-[[(1*R*)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]methoxyphosphinyl]-*L*-lactic acid, methyl ester (6)

A solution of methyl hydrogen[(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (4.5 g, 16.47 mmol) in $CH_2Cl_2$ (100 mL) was treated with 2M thionyl chloride in $CH_2Cl_2$ (9.225 mL, 18.45 mmol) and stirred for 4 h at room temperature. The solvent was removed and the residue was pumped dry in vacuo for 0.5 h to give the chloridate 5 as a yellow oil (4.8 g, 100%, crude product). This material was dissolved in $CH_2Cl_2$ (45 mL), chilled to 0-5°C under an argon atmosphere and a solution of (-)-methyl *L*-lactate (1.712 mL, 17.92 mmol), and $Et_3N$ (2.927 mL, 21.00 mmol) in $CH_2Cl_2$ (45 mL) was added dropwise. Stirred at 5°C for 15 min then stirred at RT for 5 days. The solution was concentrated to give a residue which was dissolved in EtOAc (150 mL) and washed with $H_2O$ (75 mL), sat. $NaHCO_3$ (75 mL), 3M HCl (75 mL), and brine (75 mL), then dried ($MgSO_4$), filtered and concentrated to give the crude product (5.3 g, 90%) as a tan oil. An additional reaction was performed to give a total of 9.9 g of crude product which was purified by silica gel chromatography (85:15 EtOAc:hexanes, 300 g $SiO_2$) to give pure 6 (9.0 g, 80%), as a colorless oil.

Anal.

| | C | H | N |
|---|---|---|---|
| Calc'd. for $C_{15}H_{22}NO_7P \cdot 0.22H_2O$ | 49.59 | 6.23 | 3.86 |
| Found | 49.57 | 6.15 | 3.81 |

Spectral Data

$^{1}$H Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 7.36-7.30 (m, 5H, Ph-); 5.55 (br d, 0.5H, NH); 5.15-4.92 (3m, 3.5H, Ph$\underline{CH_2}$ + $\underline{CH}$ + 0.5 $\underline{NH}$); 4.35-4.10 (2m, 1H, $\underline{CH}$); 3.82 and 3.73 (2d, 3H, J= 10.9, and 10.9); 3.76 (s, 3H); 1.56 (d, 1.5H, J= 6.9); 1.42 (m, 4.5H).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 27.71 (s); 26.83 (s).

FT - IR (neat)

Major bands: 3255, 3036, 2990, 2957, 1758, 1721, 1536, 1454, 1380, 1304, 1233, 1184, 1103, 1046, 997, 914, 859, 827, 801, 745, 699, 606, 549 $cm^{-1}$.

Mass Spectrum

Method of Ionization = DEI

Calc'd for $C_{15}H_{22}NO_7P$ = 359

Found: 359 ($m^+$), 91 (100%).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc-hexanes (85:15) | 0.41 | Homogeneous |

O-[[(1*R*)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(*L*)-lactic acid methyl ester (8)

A solution of O-[[(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]methoxyphosphinyl]-(*L*)-lactic acid methyl ester (3.0 g, 8.35 mmol) in EtOAc (150 mL) was hydrogenated at 15 psi. for 1.0 h over 10% palladium on carbon (1.5 g) (Parr hydrogenation apparatus). The suspension was filtered through a celite pad; the pad was washed with EtOAc (2 x 50 mL) and the combined filtrates were concentrated to give crude 7 as a colorless oil, which was used immediately in the next step below.

To a solution of carbobenzyloxyglycine (2.092 g, 10.00 mmol) in THF (60 mL) at 0°C under argon was added 4-methylmorpholine (1.10 mL, 10.0 mmol) followed by isobutyl chloroformate (1.30 mL, 10.0 mmol). After stirring at 0°C for 15 min, additional 4-methylmorpholine (1.10 mL, 10.0 mmol) was added followed by a solution of the crude amine 7 (prepared above) in THF (45 mL). After stirring at 0°C for 3 h, the reaction was quenched by the addition of sat. $NH_4Cl$ (9 mL). The suspension was filtered and the funnel was washed with THF (2 x 50 mL). The combined filtrate was concentrated and the residue dissolved in $CH_2Cl_2$ (150 mL) and washed with 1.0M HCl (2 x 75 mL), sat. $NaHCO_3$ (2 x 75 mL), and brine (150 mL). The $CH_2Cl_2$ layer was dried ($MgSO_4$), filtered and concentrated to give the crude product (3.5 g, 100%), which was purified on a silica gel column (92:8 EtOAc:EtOH, 105 g $SiO_2$) to give pure 8 (2.9 g, 83%) as a colorless viscous oil.

Anal.

| | C | H | 6N |
|---|---|---|---|
| Calc'd. for $C_{17}H_{25}N_2O_8P \cdot 0.6H_2O$ | 47.80 | 6.18 | 6.56 |
| Found | 47.82 | 6.15 | 6.59 |
| | 47.75 | 6.16 | 6.54 |

Spectral Data

$^{1}$H Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

δ 7.35 (m, 5H, Ph-); 7.15 and 6.65 (2m, 1H, NH); 5.49 (m, 1H, NH); 5.13 (s, 2H, Ph$\underline{CH_2}$-); 5.05 and 4.90 (2m, 1H, -OC$\underline{H}$($CH_3$)$CO_2$-); 4.62-4.54 (m, 1H, -NH$\underline{CH}$($CH_3$)P-); 3.97-3.85 (m, 2H,-NH$\underline{CH_2}$CO-); 3.82-3.73 (m, 6H, -$CO_2\underline{CH_3}$ and P-O$\underline{CH_3}$); 1.57 and 1.52 (2d, 3H, -OCH($\underline{CH_3}$)CO-); 1.42-1.38 (m, 3H, -NHCH($\underline{CH_3}$)P-).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

δ 27.49 (s); 26.26 (s).

FT - IR (thin film on KBr plate)

Major bands: 3279, 3064, 2956, 2855, 1757, 1678, 1532, 1453, 1379, 1308, 1234, 1101, 1039, 996, 859, 827, 740, 699, 586, 559, 451 $cm^{-1}$.

Mass Spectrum

Method of Ionization = DEI

Calc'd for $C_{17}H_{25}N_2PO_8$ = 416.1

Found: 416 ($m^+$), 91 (100%).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc-EtOH (92:8) | 0.43, 0.40 | Two overlapping spots (two diasteromers) |

O-[[(1*R*)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-*L*-lactic acid, lithium salt (9)

To a solution of O-[[(1*R*)-N-[N-phenylmethoxycarbonyl)-glycyl]-1-aminoethyl]methoxyphosphinyl]-*L*-lactic acid, methyl ester (100 mg, 0.24 mmol) in THF (3 mL) and deionized $H_2O$ (1.5 mL) was added a solution of $LiOH \cdot H_2O$ in deionized $H_2O$ (1.5M, 160 $\mu$L, 0.24 mmol). After stirring at room temperature for 45 min, the mixture was concentrated and the residue was coevaporated with $CH_3CN$ (2 x 15 mL) to give 9 (98 mg, 100%), as a white solid.

Spectral Data

$^1$H Nuclear Magnetic Resonance ($D_2O$)

(Mixture of two diastereomers at phosphorus). $\delta$ 7.30-7.27 (m, 5H, Ph-); 5.01 (s, 2H, $PhCH_2$-); 4.40-4.32 (m, 1H, $-OCH(CH_3)CO-$); 3.73 and 3.72 (2s, $CH_2$, $-NCH_2CO-$); 3.64 (d, 3H, J= 10.7, $-P(OCH_3)$); 3.69-3.54 (m, 1H, $-NCH(CH_3)P-$); 1.33 (dd, 3H, J= 6.6, 18,8, $-OCH(CH_3)CO-$); 1.24 (dd, 2.2H, J= 4.8, 11.6, $-NCH(CH_3)P-$, major conformer); 1.19-1.10 (m, 0.8H, $-NCH(CH_3)P-$, minor conformer).

$^{31}$P Nuclear Magnetic Resonance ($D_2O$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 27.72 (s) and 27.64 (s).

O-[[(1*R*)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-hydroxyphosphinyl]-*L*-lactic acid, monolithium N-methyl-*t*-butylammonium salt (10)

A solution of O-[[(1*R*)-N-[N-(phenylmethoxycarbonyl)-glycyl]-1-aminoethyl]methoxyphosphinyl]-*L*-lactic acid, lithium salt (88 mg, 0.216 mmol) in *tert*-butylamine (freshly distilled from KOH) (9 mL) and 1,4-dioxane (4.5 mL) was heated at reflux for 24 h under argon. After cooling to room temperature, the suspension was concentrated to give a white powder, which was dried in vacuo for 2 h to give 10 (104 mg, 100%).

Spectral Data

$^1$H Nuclear Magnetic Resonance ($D_2O$)

$\delta$ 7.30 (m, 5H, Ph-); 5.01 (s, 2H, Ph$\underline{CH_2}$-); 4.42 (m, 1H, -O$\underline{CH}(CH_3)$CO-); 3.95 (m, 1H, -N$\underline{CH}(CH_3)$P-); 3.71 (s, 2H, -N$\underline{CH_2}$CO-); 1.27 (d, 3H, J= 6.6, -OCH($\underline{CH_3}$)CO-); 1.22 (s, 12H, ($\underline{CH_3}$)$_3$C-$NH_2\underline{CH_3}$); 1.15 (dd, 2.4H, J= 7.2, 15.2, -NCH($\underline{CH_3}$)P-, major conformer); 1.05 (m, 0.6H, -NCH($\underline{CH_3}$)P-, minor conformer).

$^{31}$P Nuclear Magnetic Resonance ($D_2O$)

$\delta$ 20.64 (s).

O-[(*L*)-1-[[N-(Phenylmethoxycarbonyl)glycylamino]ethyl]-hydroxyphosphinyl]-*L*-lactic acid, dilithium salt (11)

Bio Rad AG50W-X8 ($H^+$) resin was converted to the lithium form by suspending AG50W-X8($H^+$) resin (400 g) in 1.0 M LiOH (172 g LiOH·$H_2O$ dissolved in 4 L of deionized $H_2O$) for 1.0 h, followed by suction filtration and washing of the resin with

deionized $H_2O$ until the pH of the filtrate was neutral. The moist resin was stored at 0°C in an amber bottle. A solution of O-[[(1*R*)-N-[N-(phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-hydroxyphosphinyl]-*L*-lactic acid, monolithium N-methyl-*t*-butyl-ammonium salt (98 mg, 0.203 mmol) in deionized $H_2O$ (1.0 mL) was loaded to a column of AG50W-X8 ($Li^+$) resin (10 mL bed volume, 1 x 13 cm), packed and eluted with deionized $H_2O$. Twenty fractions were collected (1.0 mL/fraction). Product found in fractions 6 and 7 (UV active and $I_2$ staining) was concentrated to a residue and coevaporated with $CH_3CN$ (2 x 2 mL) and $Et_2O$ (2 mL) to give the target 11 (50 mg, 62%), as a white solid. A portion (30 mg) was transmitted to WRAIR on July 23, 1996 (Lot No. NJ22-52-1).

Anal.

| | | C | H | N |
|---|---|---|---|---|
| Calc'd. for | $C_{15}H_{19}Li_2N_2O_8P \cdot 1.0\ H_2O$ | 42.71 | 5.11 | 6.64 |
| Found | | 42.72 | 5.12 | 6.64 |
| | | 42.67 | 5.17 | 6.59 |

Spectral Data

$^1$H Nuclear Magnetic Resonance ($D_2O$)

$\delta$ 7.31-7.27 (m, 5H, Ph-); 5.01 (s, $CH_2$, Ph$\underline{CH_2}$-); 4.42 (m, 1H, -O$\underline{CH}(CH_3)$CO-); 3.95 (m, 1H, -N$\underline{CH}(CH_3)$P-); 3.71 (s, 2H, -N$\underline{CH_2}$CO-); 1.26 (d, 3H, J= 6.8, -OCH($\underline{CH_3}$)CO-); 1.15 (dd, 2.3H, J= 7.2, 15.2, -NCH($\underline{CH_3}$)P-, major conformer); 1.04 (m, 0.7H, -NCH($\underline{CH_3}$)P-, minor conformer).

Source of Materials

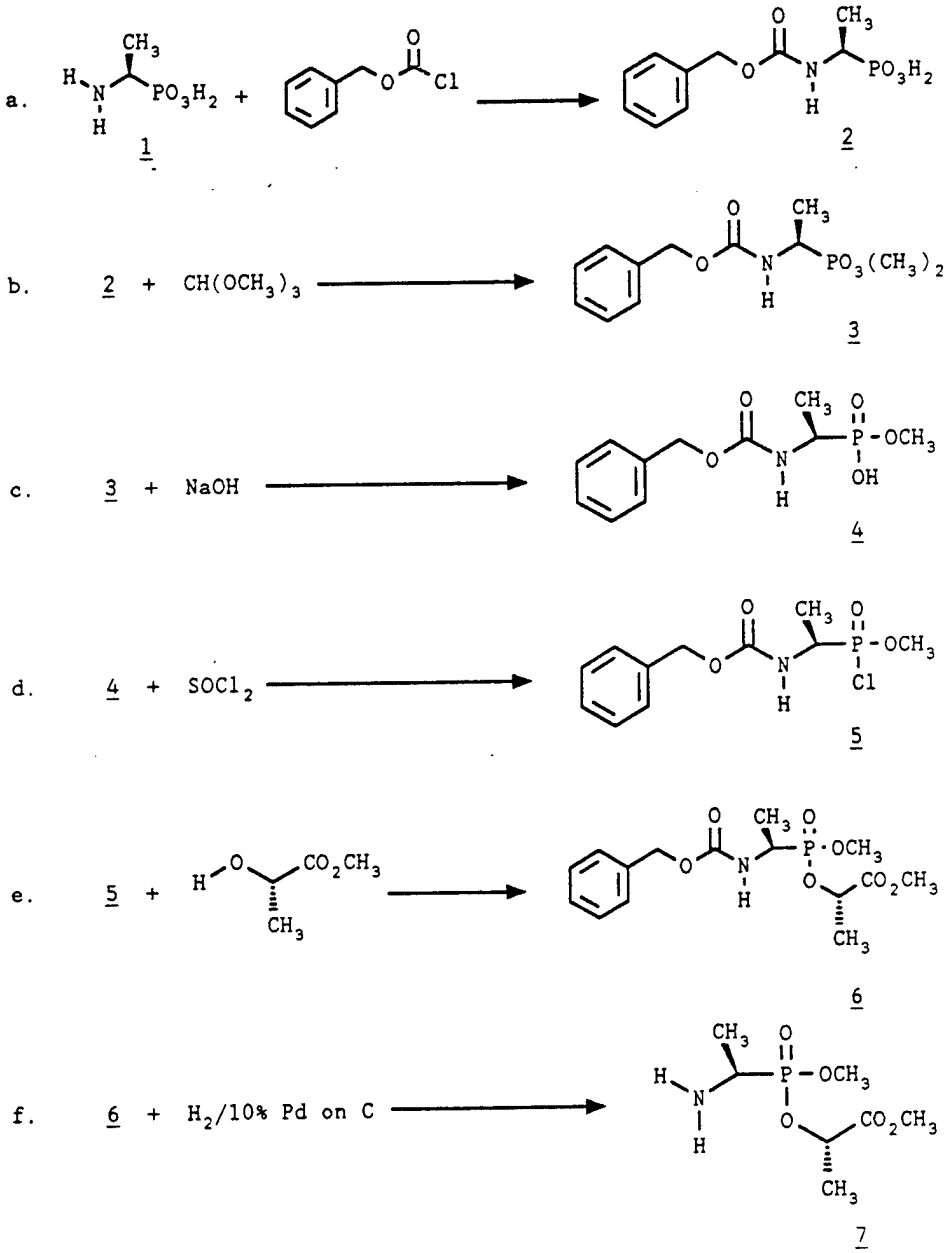
| | | |
|---|---|---|
| 1. | (*R*)-(-)-(1-Aminoethyl)-phosphonic acid | Aldrich Chemical Co., Inc. |
| 2. | Sodium hydrogen carbonate | J.T. Baker Chemical Co. |
| 3. | Sodium carbonate | J.T. Baker Chemical Co. |
| 4. | Sodium hydroxide | J.T. Baker Chemical Co. |
| 5. | Benzyl chloroformate | Aldrich Chemical Co., Inc. |
| 6. | Ether | Fisher Scientific |
| 7. | Hydrochloric acid | J.T. Baker Chemical Co. |
| 8. | Ethyl acetate | E.M. Science |
| 9. | Molecular sieves (4Å) | Aldrich Chemical Co., Inc. |
| 10. | Trimethyl orthoformate | Fluka |
| 11. | Silica gel | E.M. Science |
| 12. | Ethanol | US Industrial Chem. Corp. |
| 13. | Dichloromethane | J.T. Baker Chemical Co. |
| 14. | Methanol | J.T. Baker Chemical Co. |
| 15. | Thionyl chloride (2M in $CH_2Cl_2$) | Aldrich Chemical Co., Inc. |
| 16. | (-)-Methyl *L*-lactate | Fluka |
| 17. | Triethylamine | Aldrich Chemical Co., Inc. |
| 18. | Magnesium sulfate | J.T. Baker Chemical Co. |
| 19. | Hexanes | J.T. Baker Chemical Co. |
| 20. | 10% Palladium on carbon | Aldrich Chemical Co., Inc. |
| 21. | Hydrogen (gas) | Matheson |
| 22. | Celite | Manville |
| 23. | Carbobenzyloxyglycine | Aldrich Chemical Co., Inc. |
| 24. | Tetrahydrofuran | Aldrich Chemical Co., Inc. |
| 25. | 4-Methylmorpholine | Aldrich Chemical Co., Inc. |

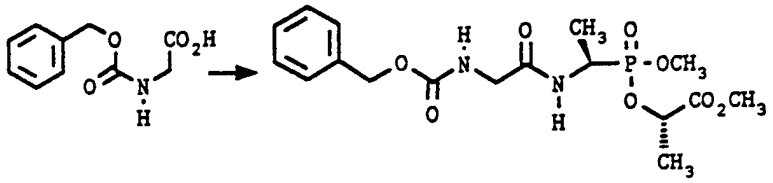
| | | |
|---|---|---|
| 26. | Isobutyl chloroformate | Aldrich Chemical Co., Inc. |
| 27. | Ammonium chloride | Aldrich Chemical Co., Inc. |
| 28. | Lithium hydroxide monohydrate | Aldrich Chemical Co., Inc. |
| 29. | Acetonitrile | Aldrich Chemical Co., Inc. |
| 30. | *tert*-Butylamine | Aldrich Chemical Co., Inc. |
| 31. | 1,4-Dioxane | Aldrich Chemical Co., Inc. |
| 32. | AG50W-X8($H^+$) resin | Bio-Rad Laboratories |
| 33. | Diethyl ether | Fisher Scientific |

19b. O-[[(*L*)-1-[[N-(Phenylmethoxycarbonyl)glycylamino]ethyl]-hydroxyphosphinyloxy]-*L*-lactic acid, dilithium salt (11)

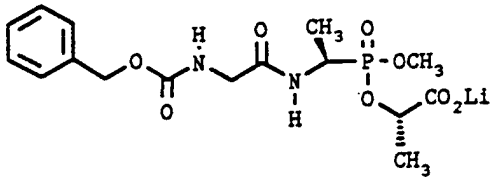
Compound 11 has been prepared by the following reaction sequence. 1.4 g of 11 was transmitted to WRAIR on August 7, 1996 (Lot No. NJ22-57-1).

Reaction Sequence

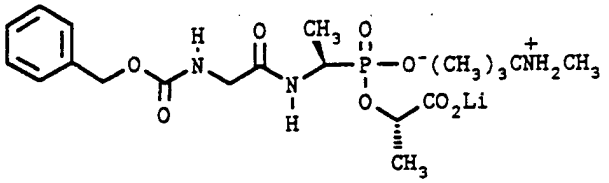


g. 7 + 

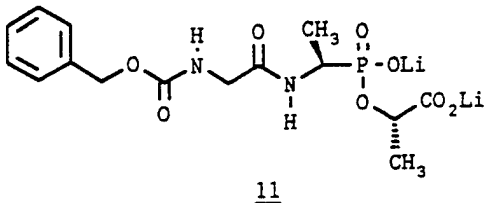
8

h. 8 + LiOH ⟶ 

9

i. 9 + tert-butylamine ⟶ 

10

j. 10 + AG50W-X8($Li^+$) ⟶ 

11

## Experimental[19-22]

### [(1*R*)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (2)

To a stirred slurry of (*R*)-(-)-(1-aminoethyl)-phosphonic-acid (1) (6.07 g, 48.5 mmol), sodium hydrogen carbonate (8.16 g, 97.1 mmol), sodium carbonate (10.3 g, 97.2 mmol), and 2N sodium hydroxide (48.8 mL, 97.6 mmol) at 5-10°C was added benzyl chloroformate (7.2 mL, 50.4 mmol) slowly by syringe. Two additional 7.2 mL portions of benzyl chloroformate were added at 1.0 h intervals, and then the mixture was stirred at room temperature for 20 h. To the white suspension was added enough 2N NaOH (15 mL) and $H_2O$ (150 mL) to completely dissolve the mixture. This aqueous solution was washed with $Et_2O$ (2 x 100 mL), acidified to pH 1-2 with conc. HCl, and then extracted with EtOAc (3 x 120 mL). The combined organic layer was washed with brine (120 mL), dried (4Å molecular sieves), filtered, and concentrated to give 2 (11.3 g, 90%) as a white foam which solidified. An additional reaction was performed to give a total of 20.0 g of 2.

### Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 10.0-9.60 (br m, 2H, -P(O<u>H</u>)$_2$); 7.26 (s, 5H, Ph-); 5.65 (br m, 1H, NH); 5.11-4.92 (m, 2H, Ph-<u>C</u>H$_2$); 4.10-3.90 (br m, 1H, -CH); 1.28-1.24 (dd, 3H, dd, 3H, $CH_3$).

Dimethyl [(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]-phosphonate (3)

A solution of [(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (8.7 g, 33.6 mmol) in trimethyl orthoformate (265 mL) was heated to reflux for 48 h. After cooling, the solution was concentrated to give a residue, which was purified by silica gel chromatography (5% EtOH/$CH_2Cl_2$, 367 g $SiO_2$) to give 3 (5.1 g, 53%) as a colorless oil which solidified on standing at 0°C. An additional reaction was performed to give a total of 10.1 g, of 3.

Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 7.35 (s, 5H, Ph-); 5.12 (s, 2H, Ph$CH_2$-); 5.00-4.95 (br d, 1H, NH); 4.25-4.15 (br m, 1H, CH); 3.75-3.73 (2s, 6H, 2 x O$CH_3$); 1.41-1.37 (dd, 3H, $CH_3$).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 28.24 (s, 1P).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

| Eluent | Rf Value | Comment |
|---|---|---|
| $CH_2Cl_2$-EtOH (19:1) | 0.32 | Homogeneous |

Methyl hydrogen[(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (4)

A solution of dimethyl [(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (5.0 g, 17.4 mmol) in $CH_3OH$ (20.5 mL) and 2N NaOH (13.5 mL, 27.0 mmol) was stirred at room temperature for 24 h. After dilution with $H_2O$ (50 mL), the solution was washed with $Et_2O$ (2 x 60 mL), acidified with 6N HCl to pH 1-2, and extracted with $CH_2Cl_2$ (4 x 100 mL). The combined $CH_2Cl_2$ was washed with brine (100 mL), dried ($Na_2SO_4$), filtered, and concentrated to give a residue which was coevaporated with $Et_2O$ (20 mL) and then dried in vacuo to give 4 (4.3 g, 90%) as a white solid, mp 119-122°C. An additional reaction was performed to give a total of 9.0 g of 4.

Spectral Data

$^1$H Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 7.33 (s, 5H, Ph-); 6.89 (m, 1H, -P(OH)); 5.73 (m, 0.2H, NH, minor conformer); 5.27 (m, 0.8H, NH, major conformer); 5.11 (s, 2H, Ph$CH_2$-); 4.16 (m, 0.8H, CH, major conformer); 4.02 (m, 0.2H, CH, minor conformer); 3.70 (d, 3H, -O$CH_3$); 1.36-132 (dd, 2.4H, J= 7.0, 16.7, $CH_3$, major conformer); 1.25 (m, 0.6H, $CH_3$, minor conformer).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

$\delta$ 28.63 (s, ~80%, major conformer); 27.89 (br, ~20%, minor conformer).

FT - IR (KBr)

Major bands: 3283, 2643, 2301, 1689, 1543, 1453, 1374, 1302, 1260, 1231, 1159, 1114, 1047, 991, 810, 696, 549 $cm^{-1}$.

O-[[(1*R*)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]methoxyphosphinyl]-(*L*)-lactic acid, methyl ester (6)

A solution of methyl hydrogen[(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (4.5 g, 16.47 mmol) in $CH_2Cl_2$ (100 mL) was treated with 2M thionyl chloride in $CH_2Cl_2$ (9.225 mL, 18.45 mol) and stirred for 4 h at room temperature. The solvent was removed and the residue was pumped dry in vacuo for 0.5 h to give the chloridate 5 as a yellow oil (4.8 g, 100%, crude product). This material was dissolved in $CH_2Cl_2$ (45 mL), chilled to 0-5°C under an argon atmosphere and a solution of (-)-methyl *L*-lactate (1.712 mL, 17.92 mmol), and $Et_3N$ (2.927 mL, 21.00 mmol) in $CH_2Cl_2$ (45 mL) was added dropwise. Stirred at 5°C for 15 min then stirred at RT for 5 days. The solution was concentrated to give a residue which was dissolved in EtOAc (150 mL) and washed with $H_2O$ (75 mL), sat. $NaHCO_3$ (75 mL), 3M HCl (75 mL), and brine (75 mL), then dried ($MgSO_4$), filtered and concentrated to give the crude product (5.3 g, 90%) as a tan oil. An additional reaction was performed to give a total of 9.9 g of crude product which was purified by silica gel chromatography (85:15 EtOAc:hexanes, 300 g $SiO_2$) to give pure 6 (9.0 g, 80%), as a colorless oil.

Anal.

| | C | H | N |
|---|---|---|---|
| Calc'd. for $C_{15}H_{22}NO_7P \cdot 0.22H_2O$ | 49.59 | 6.23 | 3.86 |
| Found | 49.57 | 6.15 | 3.81 |

Spectral Data

$^{1}$H Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 7.36-7.30 (m, 5H, Ph-); 5.55 (br d, 0.5H, NH); 5.15-4.92 (3m, 3.5H, Ph$\underline{CH_2}$ + $\underline{CH}$ + 0.5 $\underline{NH}$); 4.35-4.10 (2m, 1H, $\underline{CH}$); 3.82 and 3.73 (2d, 3H, J= 10.9, and 10.9); 3.76 (s, 3H); 1.56 (d, 1.5H, J= 6.9); 1.42 (m, 4.5H).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 27.71 (s); 26.83 (s).

FT - IR (neat)

Major bands: 3255, 3036, 2990, 2957, 1758, 1721, 1536, 1454, 1380, 1304, 1233, 1184, 1103, 1046, 997, 914, 859, 827, 801, 745, 699, 606, 549 $cm^{-1}$.

Mass Spectrum

Method of Ionization = DEI

Calc'd for $C_{15}H_{22}NO_7P$ = 359

Found: 359 ($m^+$), 91 (100%).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc-hexanes (85:15) | 0.41 | Homogeneous |

O-[[(1*R*)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(*L*)-lactic acid, methyl ester (8)

A solution of O-[[(1*R*)-N-(phenylmethoxycarbonyl)-1-aminoethyl]methoxyphosphinyl]-(*L*)-lactic acid methyl ester (3.0 g, 8.35 mmol) in EtOAc (150 mL) was hydrogenated at 15 psi. for 1.0 h over 10% palladium on carbon (1.5 g) (Parr hydrogenation apparatus). The suspension was filtered through a celite pad; the pad was washed with EtOAc (2 x 50 mL) and the combined filtrates were concentrated to give crude 7 as a colorless oil, which was used immediately in the next step below.

To a solution of carbobenzyloxyglycine (2.092 g, 10.00 mmol) in THF (60 mL) at 0°C under argon was added 4-methylmorpholine (1.10 mL, 10.0 mmol) followed by isobutyl chloroformate (1.30 mL, 10.0 mmol). After stirring at 0°C for 15 min, additional 4-methylmorpholine (1.10 mL, 10.0 mmol) was added followed by a solution of the crude amine 7 (prepared above) in THF (45 mL). After stirring at 0°C for 3 h, the reaction was quenched by the addition of sat. $NH_4Cl$ (9 mL). The suspension was filtered and the funnel was washed with THF (2 x 50 mL). The combined filtrate was concentrated and the residue dissolved in $CH_2Cl_2$ (150 mL) and washed with 1.0M HCl (2 x 75 mL), sat. $NaHCO_3$ (2 x 75 mL), and brine (150 mL). The $CH_2Cl_2$ layer was dried ($MgSO_4$), filtered and concentrated to give the crude product (3.5 g, 100%), which was purified on a silica gel column (92:8 EtOAc:EtOH, 105 g $SiO_2$) to give pure 8 (2.9 g, 83%) as a colorless viscous oil.

Anal.

| | C | H | N |
|---|---|---|---|
| Calc'd. for $C_{17}H_{25}N_2O_8P \cdot 0.6H_2O$ | 47.80 | 6.18 | 6.56 |
| Found | 47.82 | 6.15 | 6.59 |
| | 47.75 | 6.16 | 6.54 |

Spectral Data

$^{1}$H Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 7.35 (m, 5H, Ph-); 7.15 and 6.65 (2m, 1H, NH); 5.49 (m, 1H, NH); 5.13 (s, 2H, Ph$\underline{CH_2}$-); 5.05 and 4.90 (2m, 1H, -O$\underline{CH}$($CH_3$)$CO_2$-); 4.62-4.54 (m, 1H, -NH$\underline{CH}$($CH_3$)P-); 3.97-3.85 (m, 2H,-NH$\underline{CH_2}$CO-); 3.82-3.73 (m, 6H, -$CO_2\underline{CH_3}$ and P-O$\underline{CH_3}$); 1.57 and 1.52 (2d, 3H, -OCH($\underline{CH_3}$)CO-); 1.42-1.38 (m, 3H, -NHCH($\underline{CH_3}$)P-).

$^{31}$P Nuclear Magnetic Resonance ($CDCl_3$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 27.49 (s); 26.26 (s).

FT - IR (thin film on KBr plate)

Major bands: 3279, 3064, 2956, 2855, 1757, 1678, 1532, 1453, 1379, 1308, 1234, 1101, 1039, 996, 859, 827, 740, 699, 586, 559, 451 $cm^{-1}$.

Mass Spectrum

Method of Ionization = DEI

Calc'd for $C_{17}H_{25}N_2PO_8$ = 416.1

Found: 416 ($m^+$), 91 (100%).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

| Eluent | Rf Value | Comment |
|---|---|---|
| EtOAc-EtOH (92.8) | 0.43, 0.40 | Two overlapping spots (two diasteromers) |

O-[[(1*R*)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(*L*)-lactic acid, lithium salt (9)

To a solution of O-[[(1*R*)-N-[N-(phenylmethoxycarbonyl)-glycyl]-1-aminoethyl]methoxyphosphinyl]-(*L*)-lactic acid methyl ester (2.5 g, 6.04 mmol) in THF (75 mL) and deionized $H_2O$ (37.5 mL) was added a solution of $LiOH \cdot H_2O$ in deionized $H_2O$ (1.5M, 4.0 mL, 6.0 mml). After stirring at room temperature for 45 min, the mixture was concentrated and the residue was coevaporated with $CH_3CN$ (5 x 75 mL) and $Et_2O$ (100 mL) to give 9 (2.4 g, 98%), as a white solid.

Spectral Data

$^1H$ Nuclear Magnetic Resonance ($D_2O$)

(Mixture of two diastereomers at phosphorus).

$\delta$ 7.30-7.27 (m, 5H, Ph-); 5.01 (s, 2H, $PhCH_2$-); 4.40-4.32 (m, 1H, -O$CH(CH_3)CO$-); 3.73 and 3.72 (2s, $CH_2$, -$NCH_2CO$-); 3.64 (d, 3H, J= 10.7, -$P(OCH_3)$); 3.69-3.54 (m, 1H, -$NCH(CH_3)P$-); 1.33 (dd, 3H, J= 6.6, 18,8, -$OCH(CH_3)CO$-); 1.24 (dd, 2.2H, J= 4.8, 11.6, -$NCH(CH_3)P$-, major conformer); 1.19-1.10 (m, 0.8H, -$NCH(CH_3)P$-, minor conformer).

$^{31}P$ Nuclear Magnetic Resonance ($D_2O$)

(Mixture of two diastereomers at phosphorus)

$\delta$ 27.70 (s) and 27.61 (s).

O-[[(1*R*)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-hydroxyphosphinyl]-*L*-lactic acid, monolithium N-methyl-*t*-butylammonium salt (10)

A solution of O-[[(1*R*)-N-[N-(phenylmethoxycarbonyl)-glycyl]-1-aminoethyl]methoxyphosphinyl]-*L*-lactic acid, lithium salt (2.4 g, 5.88 mmol) in *tert*-butylamine (freshly distilled from KOH) (250 mL) and 1,4-dioxane (125 mL) was heated at reflux for 24 h under argon. After cooling to room temperature, the suspension was concentrated to a residue which was coevaporated with $Et_2O$ (4 x 50 mL) to give a white solid, which was dried in vacuo for 2 h to give 10 (2.8 g, 99%).

Spectral Data

$^1$H Nuclear Magnetic Resonance ($D_2O$)

$\delta$ 7.30 (m, 5H, Ph-); 5.01 (s, 2H, Ph$\underline{CH_2}$-); 4.42 (m, 1H, -O$\underline{CH}(CH_3)$CO-); 3.95 (m, 1H, -N$\underline{CH}(CH_3)$P-); 3.71 (s, 2H, -N$\underline{CH_2}$CO-); 1.27 (d, 3H, J= 6.6, -OCH($\underline{CH_3}$)CO-); 1.22 (s, 12H, ($\underline{CH_3}$)$_3$C-$NH_2\underline{CH_3}$); 1.15 (dd, 2.4H, J= 7.2, 15.2, -NCH($\underline{CH_3}$)P-, major conformer); 1.05 (m, 0.6H, -NCH($\underline{CH_3}$)P-, minor conformer).

$^{31}$P Nuclear Magnetic Resonance ($D_2O$)

$\delta$ 20.61 (s).

O-[(*L*)-1-[[N-(Phenylmethoxycarbonyl)glycylamino]ethyl]-hydroxyphosphinyl]-*L*-lactic acid, dilithium salt (11)

Bio Rad AG50W-X8 ($H^+$) resin was converted to the lithium form by suspending AG50W-X8($H^+$) resin (400 g) in 1.0 M LiOH (172 g $LiOH \cdot H_2O$ dissolved in 4 L of deionized $H_2O$) for 1.0 h, followed by suction filtration and washing of the resin with deionized $H_2O$ until the pH of the filtrate was neutral (ca. pH 7). The moist resin was stored at 0°C in an amber bottle. A solution of O-[[(1*R*)-N-[N-(phenylmethoxycarbonyl)glycyl]-1-aminoethyl]hydroxyphosphinyl]-*L*-lactic acid, monolithium N-methyl-*t*-butylammonium salt (2.8 g, 5.8 mmol) in deionized $H_2O$ (50 mL) was loaded to a column of AG50W-X8 ($Li^+$) resin (300 mL bed volume, 3 x 42 cm), packed and eluted with deionized $H_2O$. Twenty fractions were collected (15 mL/fraction). Product found in fractions 9 - 12 (UV active and $I_2$ staining) was concentrated to a residue and coevaporated with $CH_3CN$ (5 x 20 mL) and $Et_2O$ (5 x 20 mL) to give the target 11 (1.6 g, 56%), as a white solid. A portion (1.4 g) was transmitted to WRAIR on August 7, 1996 (Lot No. NJ22-57-1).

Anal.

| | C | H | N |
|---|---|---|---|
| Calc'd. for $C_{15}H_{19}Li_2N_2O_8P \cdot 1.0H_2O$ | 43.08 | 5.06 | 6.70 |
| Found | 43.04 | 5.08 | 6.77 |
| | 42.95 | 5.11 | 6.72 |

Spectral Data

$^1$H Nuclear Magnetic Resonance ($D_2O$)

$\delta$ 7.28 (m, 5H, Ph-); 4.98 (s, $CH_2$, Ph$\underline{CH_2}$-); 4.41 (m, 1H, -O$\underline{CH}(CH_3)$CO-); 3.95 (m, 1H, -N$\underline{CH}(CH_3)$P-); 3.69 (s, 2H, -N$\underline{CH_2}$CO-); 1.25 (d, 3H, J= 6.8, -OCH($\underline{CH_3}$)CO-); 1.15 (dd, 2.4H, J= 7.2, 15.2, -NCH($\underline{CH_3}$)P-, major conformer); 1.02 (m, 0.6H, -NCH($\underline{CH_3}$)P-, minor conformer).

$^{31}$P Nuclear Magnetic Resonance ($D_2O$)

$\delta$ 20.63 (s).

$^{31}$C Nuclear Magnetic Resonance ($D_2O$)

$\delta$ 179.44, 170.52, 157.76, 147.45, 135.63, 128.14, 127.75, 127.09, 71.10, 66.63, 43.05, 42.45 (d, J= 152.2), 19.66, 14.90.

FT - IR (KBr)

Major bands: 3384, 2985, 2941, 1707, 1654, 1607, 1544, 1456, 1418, 1348, 1267, 1201, 1067, 1048, 977, 885, 809, 778, 738, 698, 576, 502 $cm^{-1}$.

Mass Spectrum

Method of Ionization = FAB (Glycerol matrix)

Calc'd for $C_{15}H_{19}Li_2N_2O_8P$ = 400.1

Found: 401.1 $(m+H)^+$, 450.2 $(m+2H-CO_2+Glycerol)^+$, 493.1 $(m+H+Glycerol)^+$.

Source of Materials

| | | |
|---|---|---|
| 1. | (*R*)-(-)-(1-Aminoethyl)-phosphonic acid | Aldrich Chemical Co., Inc. |
| 2. | Sodium hydrogen carbonate | J.T. Baker Chemical Co. |
| 3. | Sodium carbonate | J.T. Baker Chemical Co. |
| 4. | Sodium hydroxide | J.T. Baker Chemical Co. |
| 5. | Benzyl chloroformate | Aldrich Chemical Co., Inc. |
| 6. | Ether | Fisher Scientific |
| 7. | Hydrochloric acid | J.T. Baker Chemical Co. |
| 8. | Ethyl acetate | E.M. Science |
| 9. | Molecular sieves (4Å) | Aldrich Chemical Co., Inc. |
| 10. | Trimethyl orthoformate | Fluka |
| 11. | Silica gel | E.M. Science |
| 12. | Ethanol | US Industrial Chem. Corp. |
| 13. | Dichloromethane | J.T. Baker Chemical Co. |
| 14. | Methanol | J.T. Baker Chemical Co. |
| 15. | Thionyl chloride (2M in $CH_2Cl_2$) | Aldrich Chemical Co., Inc. |
| 16. | (-)-Methyl *L*-lactate | Fluka |
| 17. | Triethylamine | Aldrich Chemical Co., Inc. |
| 18. | Magnesium sulfate | J.T. Baker Chemical Co. |
| 19. | Hexanes | J.T. Baker Chemical Co. |
| 20. | 10% Palladium on carbon | Aldrich Chemical Co., Inc. |
| 21. | Hydrogen (gas) | Matheson |
| 22. | Celite | Manville |
| 23. | Carbobenzyloxyglycine | Aldrich Chemical Co., Inc. |
| 24. | Tetrahydrofuran | Aldrich Chemical Co., Inc. |
| 25. | 4-Methylmorpholine | Aldrich Chemical Co., Inc. |
| 26. | Isobutyl chloroformate | Aldrich Chemical Co., Inc. |

| | | |
|---|---|---|
| 27. | Ammonium chloride | Aldrich Chemical Co., Inc. |
| 28. | Lithium hydroxide monohydrate | Aldrich Chemical Co., Inc. |
| 29. | Acetonitrile | Aldrich Chemical Co., Inc. |
| 30. | *tert*-Butylamine | Aldrich Chemical Co., Inc. |
| 31. | 1,4-Dioxane | Aldrich Chemical Co., Inc. |
| 32. | AG50W-X8($H^+$) resin | Bio-Rad Laboratories |
| 33. | Diethyl ether | Fisher Scientific |

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